Physicochemical Peculiarities of Iodine-Dimethylsulfoxide-H$_2$O Solutions and Effect on Ion Binding to Bovine Serum Albumin

K. Grigoryan and H. Shilajyan

Department of Chemistry, Yerevan State University, 1 Alex Manoogian Street, 0025 Yerevan, Armenia

Correspondence should be addressed to K. Grigoryan; kara@ysu.am

Received 27 April 2012; Accepted 6 June 2012

Academic Editors: V. Komanicky, R. Valiullin, and X.-B. Wang

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The interaction of iodine with bovine serum albumin (BSA) in dimethylsulfoxide (DMSO) aqueous solutions was studied by means of fluorescence and UV/Vis absorption spectroscopy methods. Physicochemical peculiarities of these solutions were revealed. The results showed that the tri-iodide ion formed in the 1DMSO:2H$_2$O solution caused the fluorescence quenching of BSA. The modified Stern-Volmer quenching constant $K_a$ and corresponding thermodynamic parameters, the free energy change ($\Delta G$), enthalpy change ($\Delta H$), and entropy change ($\Delta S$), at different temperatures (293, 298, and 303 K) were calculated, which indicated that the hydrophobic and electrostatic interactions were the predominant operating forces. The binding locality distance $r$ between BSA and tri-iodide ion at different temperatures was determined based on Förster nonradiation fluorescence energy transfer theory.

1. Introduction

Iodine is an element that is needed for the production of the most important organoiiodine compounds for human health thyroid hormones: thyroxine (T4) and triiodothyronine (T3). Without sufficient iodine, body is unable to synthesize these hormones and play a role in virtually all physiological functions. T4 is transported in blood, being protein-bound, principally to globulin, transthyretin, and serum albumin [1]. The absorption of inorganic-iodine, such as $I^-$, is certainly bound to serum albumin. Serum albumins, human serum albumin (HSA), and bovine serum albumin (BSA) play an important role in the transport and disposition of a wide variety of substances like metals, fatty acids, amino acids, hormones, and drugs [2–6]. Competitive interactions in the aqueous solutions containing DMSO and ions were a subject of numerous studies [7, 8]. In this paper, the interaction of iodine with BSA in DMSO aqueous solutions was studied at different temperatures by using fluorescence and UV/Vis spectroscopy methods. The binding constants and the binding locality distance are calculated, and the thermodynamic parameters of the process are proposed.

2. Methodology

The materials used are as follows. BSA and DMSO were purchased from Sigma Chemical Company (USA). Iodine was purified by sublimation. Doubly distilled water was used for the preparation of binary mixtures of 1DMSO:2H$_2$O. BSA concentration was 0.4 mg/mL, determined by electron absorption spectra in the UV region using a molar absorption coefficient $\epsilon = 36.500$ M$^{-1}$ cm$^{-1}$ ($\lambda = 280$ nm) [9].

The methods applied are the following. To perform the UV/Vis absorption measurements, we apply the following. The absorption spectra of iodine in 1DMSO:2H$_2$O solutions were recorded in the range of 250–500 nm, using 10 mm path length quartz cell. The UV/Vis measurements were performed on a spectrophotometer Specord 50PC (Germany). Ultraviolet spectra of these solutions are characterized by absorption peaks at 289 and 354 nm, indicating the formation of $I_3^-$ [10]. In DMSO-H$_2$O solutions with low content of DMSO ($X = 0.1$), iodine behaves as if it was in pure aqueous solution [11, 12].

To perform the fluorescence measurements, we apply the following. Fluorescence spectra were recorded on a Varian...
(Australia) fluorescence spectrophotometer equipped with a circulating water bath (Lauda 100). The fluorescence spectra were scanned under the following conditions: entrance slit and exit slit widths were adjusted at 5 nm, the excitation wavelength for BSA was adjusted at 280 nm, and the emission spectra were recorded in the range of 290–500 nm. The quenching experiments at different temperatures (293, 298, and 303 K) were carried out by keeping the BSA concentration constant (0.4 mg/mL) while varying the concentration of iodine in 1DMSO:2H<sub>2</sub>O (v/v) binary mixture was used. Each experiment was performed triply, and the average data were used for the analysis. Energy transfer between tri-iodide ion and BSA was determined according to the Förster nonradiative resonance energy transfer theory. The overlap of the UV/Vis absorption spectra of iodine in 1DMSO:2H<sub>2</sub>O with the fluorescence emission spectrum of BSA using Matlab 6.5 software was determined, and the energy transfer efficiency as per Förster nonradiation fluorescence energy transfer theory was calculated as well. The intrinsic fluorescence of BSA is obtained at 345 nm being excited at 280 nm. The fluorescence intensities of Trp residues of BSA in the presence of tri-iodide ion are given in Dataset Item 1 (Table).

Fluorescence quenching can occur via different mechanisms, usually classified as dynamic quenching and static quenching. In order to elucidate the fluorescence quenching mechanism, the fluorescence quenching data at different temperatures (293, 298, and 303 K) using classical Stern-Volmer equation were analyzed [13]:

\[
\frac{F_0}{F} = 1 + K_{SV} [Q] = 1 + k_q \tau_0 [Q],
\]

where \(F_0\) and \(F\) are the fluorescence intensities of BSA in the absence and presence of quencher, respectively, \([Q]\) is the concentration of quencher, \(K_{SV}\) is the Stern-Volmer quenching constant, and \(k_q\) is the quenching rate constant of the biological macromolecule. Figure 1 shows the Stern-Volmer plots of BSA fluorescence quenching by iodine at different temperatures (●) 293, (●) 298 K, and (●) 303 K, constructed by the data given in Dataset Item 2 (Table). The Stern-Volmer quenching constant \(K_{SV}\) was determined by linear regression of a plot of \(F_0/F\) against \([Q]\) (linear region). \(\tau_0\) is the average lifetime of the molecule without any quencher. When the fluorescence lifetime of the biopolymer was \(10^{-8}\) s [14], the quenching rate constant \(k_q\) at different temperatures was then calculated. The results are summarized in Dataset Item 3 (Table).

Thermodynamic parameters and the nature of binding forces are as follows. To get the binding constant, quenching data were analyzed using the modified Stern-Volmer equation:

\[
\frac{F_0}{\Delta F} = \frac{1}{f_a K_a [Q]} + \frac{1}{f_a},
\]

where \(F_0\) is the total fluorescence in the absence of quencher, \(\Delta F\) is the difference of intensities in the absence and in the presence of quencher, \(K_a\) is the Stern-Volmer quenching constant of the accessible fraction, \([Q]\) is the concentration of quencher, and \(f_a\) is the fraction of the initial fluorescence that is accessible to quencher. The modified Stern-Volmer plots for BSA quenching by iodine at different temperatures 293 K (●), 298 K (●), and 303 K (●) are shown in Figure 2, constructed by the data given in Dataset Item 4 (Table).

The thermodynamic parameters, enthalpy change (\(\Delta H\)), entropy change (\(\Delta S\)), and free energy change (\(\Delta G\)), are the main quantities to make conclusions about the binding mode. If the temperature does not vary significantly, the enthalpy change can be regarded as a constant and the value of enthalpy change and entropy change can be estimated from the van’t Hoff equation:

\[
\ln K = \frac{-\Delta H}{RT} + \frac{\Delta S}{R},
\]

where the associative binding constant \(K\) is analogous to the effective quenching constant \(K_a\) at the corresponding temperature. The free energy change (\(\Delta G\)) can be estimated from the following relationship:

\[
\Delta G = \Delta H - T\Delta S.
\]

The thermodynamic parameters were calculated from van’t Hoff plot for BSA-iodine system shown in Figure 3 (Dataset Item 5 (Table)).

Then we have the energy transfer between tri-iodide and BSA. The distance between the donor and the acceptor can be calculated according to Förster nonradiation fluorescence energy transfer theory [15]. The efficiency of energy transfer, \(E\), is calculated by using

\[
E = 1 - \frac{F}{F_0} = \frac{R_0^6}{r^6},
\]

where \(F_0\) and \(F\) are the fluorescence intensities of BSA in the absence and presence of iodine, respectively, \(r\) is the distance between the donor and acceptor, and \(R_0\) is the critical distance.
when energy transfer efficiency is 50% and can be calculated by
\[ R_0^6 = 8.79 \times 10^{-25} k^2 n^{-4} \Phi J, \] (6)
where \( K^2 \) is the space factor of orientation, \( n \) is the refractive index of the medium, \( \Phi \) is the fluorescence quantum yield of the donor, and \( J \) is the overlap integral of the fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor. Therefore,
\[ J = \frac{\sum f(\lambda) \varepsilon(\lambda) \lambda^4 \Delta \lambda}{\sum F(\lambda) \Delta \lambda}, \] (7)
where \( F(\lambda) \) is the fluorescence intensity of the donor at the wavelength \( \lambda \) and \( \varepsilon(\lambda) \) is the molar absorption coefficient of the acceptor at the wavelength \( \lambda \). The data for the overlap integrals, Förster radius \( (R_0) \), distance between the donor and acceptor \( (r) \), and efficiency of energy transfer \( (E) \) for BSA-iodine system are given in Dataset Item 6 (Table), when \( K^2 = 2/3, n = 1.336, \) and \( \Phi = 0.15 \) [15].

3. Dataset Description

The dataset associated with this Dataset Paper consists of 7 items which are described as follows.

Dataset Item 1 (Table). Fluorescence intensities of BSA at the presence of iodine at different temperatures (293, 298, and 303 K). In the first column is given the concentration of the quencher \([Q]\) \((10^5 \text{ mol L}^{-1})\). In the second, third, and fourth columns are collected the fluorescence intensities of BSA at 293, 298, and 303 K temperatures (arbitrary units (a.u.)).

- Column 1: \([Q]\) \((\text{mol L}^{-1})\)
- Column 2: Intensity at 293 K (a.u.)
- Column 3: Intensity at 298 K (a.u.)
- Column 4: Intensity at 303 K (a.u.)

Dataset Item 2 (Table). Collected data for \( F_0/F \) of BSA quenching at the presence of iodine at different temperatures (293, 298, and 303 K). In the first column is given the concentration of the quencher \([Q]\) \((10^5 \text{ mol L}^{-1})\). In the second, third, and fourth columns are collected the data for \( F_0/F \) at 293, 298, and 303 K temperatures.

- Column 1: \([Q]\) \((\text{mol L}^{-1})\)
- Column 2: \( F_0/F \) at 293 K
- Column 3: \( F_0/F \) at 298 K
- Column 4: \( F_0/F \) at 303 K

Dataset Item 3 (Table). Temperature effect on Stern-Volmer quenching constants for the interaction of iodine with BSA. In the first column is given the temperature; in the second, Stern-Volmer quenching constants \( K_{sv} \) \((\times 10^4 \text{ L mol}^{-1})\); in the third, the quenching rate constant \( k_q \) \((\times 10^2 \text{ L mol}^{-1} \text{ s}^{-1})\); in the fourth, \( R \), which is the correlation coefficient for \( K_{sv} \) and \( k_q \).

- Column 1: Temperature (K)
- Column 2: \( K_{sv}\) (L mol\(^{-1}\))
- Column 3: \( k_q\) (L mol\(^{-1}\) s\(^{-1}\))
- Column 4: \( R \)

Dataset Item 4 (Table). \( F_0/(F_0 - F) \) for BSA quenching at the presence of iodine at different temperatures (293, 298, and 303 K). In the first column is given the concentration of the quencher \([Q]\) \((10^{-5} \text{ mol L}^{-1})\). In the second, third, and fourth columns are collected the data for \( F_0/(F_0 - F) \) at 293, 298, and 303 K temperatures.

- Column 1: \([Q]\) \((\text{mol L}^{-1})\)
- Column 2: \( F_0/(F_0 - F) \) at 293 K
- Column 3: \( F_0/(F_0 - F) \) at 298 K
- Column 4: \( F_0/(F_0 - F) \) at 303 K

Dataset Item 5 (Table). Temperature dependence of modified Stern-Volmer association constants. In the first column are given the temperatures. In the second column are given the reverse temperatures \(1/T\) \((\times 10^3 \text{ K}^{-1})\). In the third column are collected \( K_{sv} \) \((\times 10^4 \text{ L mol}^{-1})\), determined from Figure 2; in the fourth, \( R \), which is the correlation coefficient for \( K_{sv} \). In the fifth column are presented \( \ln K_{sv} \).

- Column 1: Temperature (K)
- Column 2: \(1/T\) (K\(^{-1}\))
- Column 3: \( K_{sv}\) (L mol\(^{-1}\))
- Column 4: \( R \)
- Column 5: \( \ln K_{sv}\)

Dataset Item 6 (Table). Thermodynamic parameters \( \Delta H, \Delta S, \) and \( \Delta G \) of iodine-BSA interactions. In the first column are given the temperatures. In the second and fourth columns are given \( \Delta H \) \((\text{kJ mol}^{-1})\) and \( \Delta S \) \((\text{J mol}^{-1} \text{ K}^{-1})\), which were determined from Figure 3, van’t Hoff plot \((\ln K_{sv} = -\Delta H/RT + \Delta S/R)\). In the third column are given the values of \( \Delta G \) \((\text{kJ mol}^{-1})\), which were calculated using \( \Delta G = \Delta H - T \Delta S \) equation.

- Column 1: Temperature (K)
- Column 2: \( \Delta H \) (kJ mol\(^{-1}\))
- Column 3: \( \Delta G \) (kJ mol\(^{-1}\))
- Column 4: \( \Delta S \) (J mol\(^{-1}\) K\(^{-1}\))

Dataset Item 7 (Table). Collected data of overlap integrals \((J)\), Förster radius \((R_0)\), distance between the donor and
4. Concluding Remarks

Tri-iodide ion is formed in 1DMSO:2H₂O solution, which causes the fluorescence quenching of BSA. Interactions in this system are driven mainly by hydrophobic and ionic interactions.

Dataset Availability

The dataset associated with this Dataset Paper is dedicated to the public domain using the CC0 waiver and is available at http://dx.doi.org/10.7167/2013/534328/dataset.

References

[1] G. W. Gribble, "Naturally occurring organohalogen compounds—a comprehensive survey," Progress in the Chemistry of Organic Natural Products, vol. 68, pp. 1–423, 1996.
[2] K. Grigoryan and A. Ghazaryan, "Characterization of the platinum (II) dimethylsulfoxide complex binding to bovine serum albumin by fluorescence spectroscopy method," Global Journal of Analytical Chemistry, vol. 5, pp. 1–8, 2012.
[3] K. R. Grigoryan, M. G. Aznauryan, N. A. Bagramyan, L. G. Gevorkyan, and S. A. Markaryan, "Spectroscopic determination of binding between human serum albumin and a platinum(II) dimethylsulfoxide complex," Journal of Applied Spectroscopy, vol. 75, no. 4, pp. 593–596, 2008.
[4] S. Cui, X. Hu, and J. Liu, "Study of the binding of herbacetin to bovine serum albumin by fluorescence spectroscopy," Journal of Solution Chemistry, vol. 40, no. 5, pp. 764–774, 2011.
[5] Y. Z. Zhang, B. Zhou, Y. X. Liu, C. X. Zhou, X. L. Ding, and Y. Liu, "Fluorescence study on the interaction of bovine serum albumin with P-aminoazobenzene," Journal of Fluorescence, vol. 18, no. 1, pp. 109–118, 2008.
[6] H. Xu, Q. Liu, Y. Zuo, Y. Bi, and S. Gao, "Spectroscopic studies on the interaction of vitamin C with bovine serum albumin," Journal of Solution Chemistry, vol. 38, no. 1, pp. 15–25, 2009.
[7] K. R. Grigoryan and A. A. Shiladzhyan, "The effect of solvated ions on the thermal denaturation of human serum albumin in water-dimethylsulfoxide solutions," Russian Journal of Bioorganic Chemistry, vol. 35, no. 5, pp. 581–584, 2009.
[8] S. Markarian, K. Grigoryan, and R. Harutunyan, "Physicochemical properties of CuCl₂, Cu(NO₃)₂, and Al(NO₃)₃ dimethylsulfoxide and dimethylsulfoxide-water solutions," Russian Journal of Physical Chemistry, vol. 69, pp. 990–993, 1995.
[9] A. K. Singh and M. Darshi, "A fluorescence study of (4-((1E,3E)-4-[4-(dimethylamino)phenyl]-buta-1,3-dienyl)phenyl)methanol and its bioconjugates with bovine and human serum albumins," New Journal of Chemistry, vol. 28, no. 1, pp. 120–126, 2004.
[10] Y. J. Wei, C. G. Liu, and L. P. Mo, "Ultraviolet absorption spectra of iodine, iodide ion and triiodide ion," Spectroscopy and Spectral Analysis, vol. 25, no. 1, pp. 86–88, 2005, Article in Chinese, Abstract in English.
[11] S. Markarian, R. Harutunyan, V. Grigoryan, and N. Beyleryan, "Physicochemical properties of Dialkylsulfoxide Aqueous and CCl₄ solutions," Chem. Chem. Techn., vol. 28, pp. 18–22, 1985 (Russian).
[12] D. Martin and H. G. Hauthal, Dimethylsulfoxide, Van Nostrand Reinhold, Workingham, Berkshire, UK, 1975.
[13] J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Springer, New York, NY, USA, 3rd edition, 2006.

[14] J. R. Lakowicz and G. Weber, "Quenching of fluorescence by oxygen. A probe for structural fluctuations in macromolecules," *Biochemistry*, vol. 12, no. 21, pp. 4161–4170, 1973.

[15] R. Punith, A. H. Hegde, and S. Jaldappagari, "Binding of an anti-inflammatory drug lornoxicam with blood proteins: insights from spectroscopic investigations," *Journal of Fluorescence*, vol. 21, pp. 487–495, 2011.
