Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Pharmaceutical and Biomedical Analysis 40 (2006) 1198–1201
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Determination of bovine serum albumin by a resonance light-scattering technique with the mixed-complex La(Phth)(phen)$^{3+}$

Ling Li *, Gongwu Song, Guangrong Fang

Faculty of Chemistry and Material Science, Hubei University, Wu Han 430062, PR China

Received 14 August 2005; accepted 9 September 2005
Available online 19 October 2005

Abstract

A mixed-complex of La(Phth)(phen)$^{3+}$ was synthesized. Resonance light-scattering characteristics of interaction between La(Phth)(phen)$^{3+}$ with bovine serum albumin (BSA) were studied. When BSA was added, aggregation of La(Phth)(phen)$^{3+}$ on the molecular surface of bovine serum albumin occurred in the pH 5.5–6.3, resulted in an enhanced resonance light-scattering (RLS) peak at 360 nm. The intensity of resonance light-scattering was found to be proportional to the concentration of BSA.

Keywords: Mixed-complex; Resonance light-scattering; BSA; Aggregation

The nature and dynamics of binding small molecules to biopolymers represents an area of active investigation [1]. The development of novel methods and new technique of protein determination is very important in both clinical and laboratory tests. Kinds of methods have been established to determine the content of proteins in samples [2–5]. The resonance light-scattering technique, using a common spectrofluorometer, has been developed as a sensitive instrumental analysis method in the application of determination of microamounts of biomachromolecurlers [6,7] recently. Resonance light-scattering technique was based on the fact that the aggregation of dye chromophore on the biological macromolecule can give rise to strong RLS, Ma et al. [8–10]. There is a linear relationship between the enhanced intensity and the concentration of bovine serum albumin.

On the other hand, it has been reported that mixed complexes of lanthanide series ions with phen and bpy were found to have antibacterial activity [11,12]. The complexes of lanthanide series ions with *o*-phthalic acid (Phth) were found to have fluorescence [13]. Therefore, La(Phth)(phen)$^{3+}$ was synthesized and resonance light-scattering technique with the mixed-complex La(Phth)(phen)$^{3+}$ for protein determination was experimented.

1. Experimental

1.1. Apparatus

The complex was synthesized with a GSP-77-03 magnetic mixer (Jiangsu, China). The resonance light-scattering spectrum and the intensity of resonance light scattering were measured with a Shimadzu RF-540 spectrofluorometer (Kyoto, Japan). A WH-2 vortex mixer (Huxi Instrumental Co., Shanghai, China) was used to blend the solution, and a PHB-4 pH meter (Leici Instrumental Co., Shanghai, China) was used to measure the pH value of the solution.

1.2. Reagents

All reagents for synthesis were of analytical-reagent grade, made in China.

The working solution of La(Phth)(phen)$^{3+}$ was 1.1×10^{-3} mol L^{-1}.

The stock solution of BSA was prepared by dissolving commercially purchased calf thymus BSA (Beijing Shuangxuan biological culture medium plant, China) in doubly distilled water at 0–4 °C. The working solution of the bovine serum albumin was 1.05 g L^{-1}.

Tris–HCl solution was used to control the acidity, while 0.1 mol L^{-1} NaCl was used to adjust the ionic strength of the aqueous solutions.

Doubly distilled water was used throughout.

* Corresponding author.
E-mail address: waitingll@yahoo.com (L. Li).

0731-7085/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2005.09.004

1.3. Synthesis of the complex

A certain Larrous sulfate (La^{3+}) was dissolved in some anhydrous ethanol, and equimolar phen with $LaCl_3$ was added, then stirring to dissolve phen. Ten minutes later, equimolar Phth was added, stirring for 1 h, allowed to stand for 12 h. The product was isolated by filtration and vacuum dried, then the deep red powder crystal could be got.

1.4. Standard procedure

Appropriate working solution of bovine serum albumin and 0.2 mL of La(Phth)(phen)$^{3+}$ solution, 0.4 mL Tris–HCl solution were added to a 10 mL volumetric flask. The mixture was diluted with water to 10 mL, and vortexed. Five minutes later, all the absorption and RLS measurements were obtained against the blank treated in the same way without bovine serum albumin.

The RLS spectrum was obtained by scanning simultaneously the excitation and emission monochromator of the RF-540 spectrofluorometer through the wavelength range 300–600 nm with $\Delta\lambda = 0$ nm. The RLS intensity was measured at the maximum wavelength 360 nm.

2. Results and discussion

2.1. Spectral characteristics

Fig. 1 was obtained according to the standard procedure. Fig. 1 showed that La(Phth)(phen)$^{3+}$ nearly had no RLS peaks at 400 and 360 nm. When BSA was added, enhanced RLS peaks could be observed at 400 and 360 nm, which mainly resulted from the aggregation of La(Phth)(phen)$^{3+}$ on the molecular surface of bovine serum albumin [14]. The extent to which a particle absorbs and scatters light depends on its size, shape, and index of refraction relative to the surrounding medium, and the scattering due to each sphere is proportional to the square of the volume, so the amount of scattering is directly proportional to the volume of each sphere. Thus, the larger the aggregating, the greater the scattering.

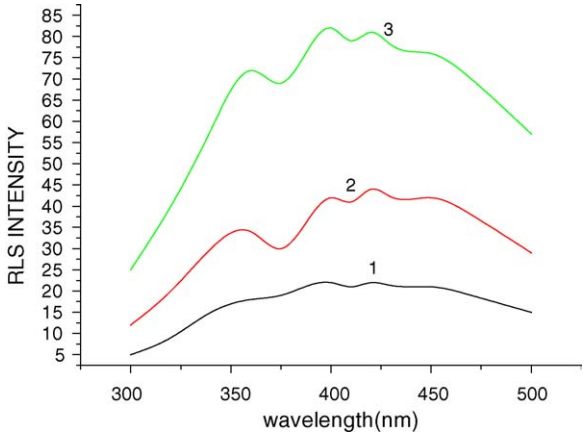

Fig. 1. Resonance light-scanning of La(phen)(Phth)$^{3+}$-BSA system. La(phen)(Phth)$^{3+}$: 3.6×10^{-5} mol L^{-1}; BSA: (1) 0.000 mg L^{-1}; (2) 0.0336 g L^{-1}; (3) 0.0672 g L^{-1}; pH 6.32.

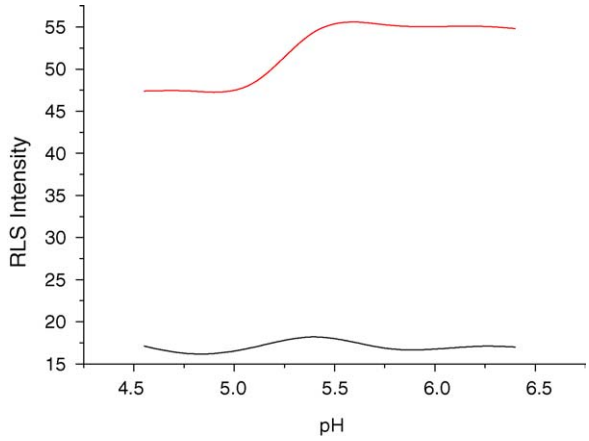

Fig. 2. Effect of pH on the intensity of RLS: (1) La(Phth)(phen)$^{3+}$: 3.6×10^{-5} mol L^{-1}; (2) La(Phth)(phen)$^{3+}$: 3.6×10^{-5} mol L^{-1}; BSA: 33.6 mg L^{-1}.

2.2. Effect of pH

Using Tris–HCl solution to control the acidity according to the procedure, the intensity of RLS in different acidity was determined. Fig. 2 showed that the RLS intensity changed a little with different acidity. However, the RLS intensity enhanced much when BSA was added. The optimal pH value seemed to relate to the isoelectric point of protein [15]. The isoelectric point of many proteins was nearly at 5, then when pH value was higher than 5.0, BSA was present with the form of anion, electrostatic attraction occurred between BSA and La(Phth)(phen)$^{3+}$, which strengthened the extend of aggregation. It can be seen that the maximum RLS intensity is obtained in the pH range 5.5–6.3, and the most maximum is in the pH 6.3, so pH 6.3 was chosen for the assay and the optimum volume of the buffer is 0.4 mL.

2.3. Effect of ionic strength

A 0.1 mol L^{-1} NaCl solution was used to adjust the ionic strength of the system, then Fig. 3 was obtained. It could be

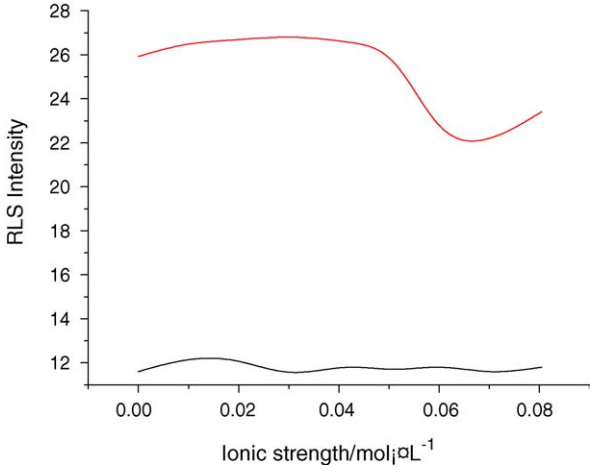

Fig. 3. Effect of electrolyte on the intensity of RLS. La(Phth)(phen)$^{3+}$; 3.6×10^{-5} mol L^{-1}; BSA: 33.6 mg L^{-1}; pH 6.32.

Table 1
Effect of concentration of La(Phth)(phen)$^{3+}$on the linear relationship

Concentration of La(Phth)(phen)$^{3+}$ (mol L^{-1})	Linear regression equation	Linear range (mg L^{-1})	Correlation coefficient
1.8×10^{-5}	$I = 1.2674 + 8.55C$	8.6–34.4	0.9995
4.2×10^{-5}	$I = 0.9977 + 16.26C$	4.3–34.4	0.9990
6.7×10^{-5}	$I = 0.9708 + 11.96C$	2.9–51.6	0.9996
9.1×10^{-5}	$I = 1.2871 + 12.37C$	0.12–67.2	0.9998

seen that RLS intensity of La(Phth)(phen)$^{3+}$ changed little with ionic strength, while BSA was added, the system was scarcely affected when ionic strength was lower than 0.05 mol L^{-1}. But with the increase of ionic strength, the RLS intensity decreased a lot. Therefore, the system can only be allowed at low ionic strength.

2.4. Effect of concentration of La(Phth)(phen)$^{3+}$

The experiment showed that the enhanced intensity of RLS took on an excellent linear relationship when the concentration of BSA was lower than 50.4 mg L^{-1} and concentration of La(Phth)(phen)$^{3+}$ was in the range of 1.8×10^{-5} to 9.1×10^{-5} mol L^{-1}. The effect of different concentration of La(Phth)(phen)$^{3+}$ on the linear relationship in the range was displayed in Table 1. It is clear that when the concentration of La(Phth)(phen)$^{3+}$ was 9.1×10^{-5} mol L^{-1}, the linear range was widest, and the correlation coefficient of the similar linear regression equation was the best. Then 9.1×10^{-5} mol L^{-1} was selected.

2.5. Calibration curve

The calibration curve was obtained according to the above standard procedure. There were linear relationship between the RLS intensity and the concentration of bovine serum albumin when the concentration of bovine serum albumin was in the range of 0.12–67.2 mg L^{-1}. The linear regression equation is

$$I = 1.2871 + 12.37C(\text{BSA}), \quad r = 0.9998$$

the limit of determination of determination (3σ) was 0.040 mg L^{-1}.

2.6. Tolerance of foreign substances

The influence of foreign coexisting substances such as metal ions and Glucose, glycin, L-cysteine were tested according to the standard procedure. The results shown in Table 2, these foreign substances had little effects on the determination of BSA.

2.7. Analysis

Three synthetic samples, prepared based on the interferences of foreign substances (Table 2), were analyzed. As Table 3 showed, the results were reproducible and reliable.

Table 2
Interferences of foreign substances

No	Foreign substances	Concentration (mg L^{-1})	Change of ΔI_{RLS} (%)
1	Zn^{2+}	0.5	0.3
2	Cu^{2+}	6.4	5.2
3	K^{+}	160	3.6
4	Ba^{2+}	160	0.6
5	Fe^{3+}	10.2	−5.6
6	As^{5+}	2.6	4.7
7	Cr^{3+}	16	−1.6
8	Pb^{2+}	12.8	2.5
9	Sn^{4+}	160	5.9
10	Hg^{2+}	3.2	−6.5
11	Glucose	123	−6.5
12	glycin	16	−2.0
13	L-cysteine	0.4	−2.3
14	Pb^{2+}	12.8	2.5

Table 3
Determination result of synthetic samples

Sample	Concentration (mg L^{-1})	Foreign substances	Found (mg L^{-1})	Recovery (%)
BSA	16.8	Zn^{2+}, Ba^{2+}, Pb^{2+}	17.3	103.3
BSA	16.8	K^{+}, glycin	17.2	102.3
BSA	16.8	Glucose, L-cysteine	16.1	95.8

0.1 mg L^{-1} Zn^{2+}, 5 mg L^{-1} Ba^{2+}, 1 mg L^{-1} Pb^{2+}, 8 mg L^{-1} K^{+}, 1 mg L^{-1} glycin, 5 mg L^{-1} Glucose, 0.1 mg L^{-1} L-cysteine.

Acknowledgement

This project was supported by The China Association for Instrument Analysis and all authors here expressed their deep thanks.

References

[1] Challa V. Kumar, Jacqueline K. Barton, Nicholas J. Turro, Photophysics of ruthenium complexes bound to double helical DNA [J], J. Am. Chem. Soc. 107 (1985) 5518–5523.
[2] T. Zor, Z. Selinger, Linearization of the bradford protein assay increases its sensitivity: theoretical and experimental studies, Anal. Biochem. 236 (1996) 302–308.
[3] X.W. Zhang, Experimental Hand Book for Biomacromolecules, Jilin University Press, Changchun, China, 1991, pp. 323–324.
[4] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the folin phenol reagent [J], J. Biol. Chem. 193 (1951) 265–271.
[5] Y.T. Wang, F.L. Zhao, K.A. Li, S.Y. Tong, Microdetermination of proteins by enhanced Rayleigh light scattering spectroscopy with morin, Fresenius J. Anal. Chem. 364 (1999) 560–565.
[6] R.F. Pasternack, C. Bustamante, P.J. Collings, A. Giannetto, E.J. Gibbs, Porphyrin assemblies on DNA as studies by a resonance light-scattering technique, J. Am. Chem. Soc. 115 (1993) 5393–5399.
[7] R.F. Pasternack, K.S. Schaefer, P. Hambright, Resonance light-scattering studies of porphyrin diacid aggregates, Inogr. Chem. 33 (1994) 2062–2065.
[8] C.Q. Ma, K.A. Li, S.Y. Tong, Enhancement of Rayleigh light scattering of acid chrome blue K by proteins and protein assay by the scattering technique [J], Analyst 122 (1997) 361.
[9] C.Q. Ma, K.A. Li, S.Y. Tong, Microdetermination of proteins by resonance light scattering spectroscopy with bromophenol blue [J], Anal. Biochem. 239 (1996) 86.

[10] C.Q. Ma, K.A. Li, S.Y. Tong, Microdetermination of proteins by Rayleigh light scattering technique with acid green 25, Anal. Chim. Acta 338 (1997) 255.
[11] X. Wang, L. Zhen, X. Su, Syntheses and characterization of rare Earth complex with biprydine and phenanthroline [J], J. East China Univ. Sci. Technol. 22 (1996) 103–106.
[12] X. Wang, H. Qing, Antibacterial experiment of the complex of samarium with phenanthroline and bipyridyl [J], J. Anhui Inst. Mach. Electron. Eng. 2 (1994) 26–28.
[13] Y. Zhao, B. Sun, J. Wu, Z. Xu, S. Yao, W. Zhou, Fluorescence and FT-irspectroscopic studies on the mixed solid complexes of europium and terbium phthalate [J], Spectrosc. Spectral Anal. 17 (1997) 82–99.
[14] C. Huang, Y. Li, N. Li, H. Luo, X. Huang, Long range assembly of nile blue sulphate on the molecular surfaces of bovine serum albumin and the determination of bovine serum albumin by triple wavelength resonance light-scattering technique [J], Chin. J. Anal. Chem. 27 (1999) 1241–1247.
[15] C. Huang, Y. Li, P. Feng, Determination of proteins with α,β,γ,δ-tetrakis(4-sulfophenyl)porphine by measuring the enhanced resonance light scattering at the air/liquid interface, Anal. Chim. Acta 443 (2001) 73.