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BIOPHYSICAL CHEMISTRY

A Novel Investigation of the Interaction of Proteins with Sodium Dodecylbenzyl Sulfonate[1]

Hong-Wen Gao*, Rong Shen, and Ya-Lei Zhang***

**State Key Laboratory of Pollution Control and Resource Reuse, School of Environmental Science and Engineering, Tongji University, Shanghai, 200092, P. R. China*

*** Anhui Professional Technology Institute, Hefei, 230051, P. R. China*

e-mail: hwgao@mail.tongji.edu.cn

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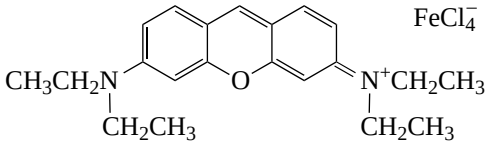
Abstract—The microsurface adsorption–spectral correction (MSASC) technique was applied to the interactions of sodium dodecylbenzyl sulfonate (SDBS) with pyronin B (PRB) and three kinds of proteins: bovine serum albumin (BSA), myoglobin (Mb), and ovalbumin (OVA). The aggregations obeyed the Langmuir isothermal adsorption. The characterization of the SDBS–PRB and SDBS–protein aggregates was performed. The break point approach was applied to examine the binding constants obtained by the MSASC technique. Results showed that the products PRB · SDBS, $SDBS_{110}$ · BSA, $SDBS_{69}$ · OVA, and $SDBS_{23}$ · Mb are formed at pH 2.81 at 30°C. The aggregation of SDBS on proteins is applied to analysis of samples with satisfactory results.

INTRODUCTION

Many chemists are always greatly interested in the assembly of small molecules on a biomacromolecule [1–5]. Thus, a stain is often used as a spectral probe in studying the interaction of small molecules with a macromolecule, especially nonspectral or weak-spectral molecules (for example, drugs, surfactants, pesticides, and toxicants). Accurate analysis of this interaction is very helpful in understanding the chemical actions that occur in a biological body and then designing a new drug or removing a toxicant from the body. Understanding this physicochemical interaction can help us to investigate further the interaction between macromolecules, e.g., polymers with proteins [6–8] and proteins with DNA [9, 10], and thus to repair DNA and develop new kinds of anticarcinogens. Molecular spectrometry is widely used, and the Pesavento [11] and Scatchard models [12] are classical to characterizing an assembly product [22]. Recently, the microsurface adsorption–spectral correction (MSASC) technique has been applied to the interaction of stains with macromolecules [18–20]. In this work, we further study its application to the aggregation of a nonspectral compound on a macromolecule. In addition, the break point approach [21] is applied to the characterization of the assembly product. The interaction of sodium dodecylbenzyl sulfonate (SDBS) with the proteins bovine serum albumin (BSA), myoglobin (Mb), and ovalbumin (OVA) with pyronin B (PRB) as the spectral probe is investigated in detail as an example. This updated approach is accurate and easy to perform. The structure of PRB is given below:



(Pyronin B, Cl 45010)

It forms a bivalent cation because of both the protonation of the left tertiary amine and the isomerization of the left phenyl in an acidic medium and monovalent cation in neutral solution. Certainly, the anions can be adsorbed on an anionic surfactant, e.g., SDBS. Similarly, the protonation of amine in protein may itself carry many positive charges, so SDBS can be attracted onto it. The aggregation of PRB on SDBS and assembly of SDBS on protein obeyed the Langmuir isothermal adsorption. The characterization of the aggregates was done using the MSASC and the break point approach. The aggregates PRB · SDBS, $SDBS_{110}$ · BSA, $SDBS_{69}$ · OVA, and $SDBS_{23}$ · Mb were formed at pH 2.81 and room temperature. The equilibrium constants of formation of the aggregates were also determined. The aggregation of SDBS on protein was applied to the quantitative detection of protein in samples with satisfactory results.

PRINCIPLE

MSASC technique. If we look at Fig. 1, the charged surfactant (S) monomer (*1*) can attract an oppositely charged stain probe (L) (*2*), but the aggregation of L on S also obeys the Langmuir isotherm

[1] This article was submitted by the authors in English.

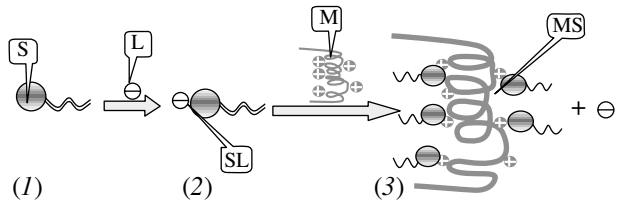


Fig. 1. The aggregation of L on an S monomer (*1*, *2*), the substitution of L, and the assembly of S on protein (M) (*3*).

adsorption. The synergism of a surfactant, e.g., solubilization, stabilization, and sensitivity enhancement was proposed earlier in, e.g., synergism perturbation [22], hydrogen bond formation [23], micelle catalysis [24], electrostatic field aggregate [25], and others. A microsurface adsorption mechanism has been developed on the basis of these models. It unites and improves both the micelle catalysis and electrostatic field aggregate theories. Its combination with the MSASC spectral correction technique [26–29] provides a very helpful experimental strategy for studying the physicochemical interaction between molecules.

If we look at the drawing in Fig. 1, the stain probe (L) can be attracted onto an oppositely charged surfactant (S) (*1*) to form an aggregate, SL, and the assembly obeys the Langmuir isotherm adsorption [30]. The S–L solution equilibrium occurs as follows (m.s. means microsurface phase):

	L	+ S (m.s.)	⟶ SL_n (m.s.)
Initial state	c_{L0} (A_0, A_0')	c_{S0}	0
Equilibrium	c_L	0	$c_{S0}(A_c)$

The Langmuir isotherm equation is expressed as

$$\frac{1}{\gamma} = \frac{1}{N} + \frac{1}{KNc_L} = a + b/c_L, \quad (1)$$

where K is the equilibrium constant and c_L is the molarity of the excess L. γ is the molar ratio of the effective L adsorbed on S. With an increase in L concentration, γ approaches the maximum binding number, N. Both N and K may be calculated by plotting line γ^{-1} vs. c_L^{-1}. Both c_L and γ are calculated by the relations [26–29]

$$\gamma = n = \eta c_{L0}/c_{S0}, \quad (2)$$

$$c_L = (1 - \eta)c_{L0}, \quad (3)$$

where

$$\eta = (A_c - \Delta A)/A_0, \quad (4)$$

where both c_{S0} and c_{L0} are the molarities of S and L in the initial solution and η indicates the effective fraction of L. A_c, A_0, and ΔA are the real absorbance of the S–L product, the measurement absorbance of L against water, and that of the S–L solution against the reagent blank directly measured at the peak wavelength λ_2, respectively. A_c is calculated by means of

$$A_c = (\Delta A - \beta\Delta A')/(1 - \alpha\beta), \quad (5)$$

where $\Delta A'$ indicates the absorbance of the S–L solution measured at the valley wavelength λ_1. Both α and β are correction constants, and they are calculated by measuring SL_n and the L solution directly.

In the reaction above, the addition of biomacromolecule (M) will result in the replacement of L and the formation of a new aggregate, MS. This process is shown in Fig. 1 (*3*) and is expressed as follows:

	SL_n	+ M (m.s.)	⟶ MS_m (m.s.)	+ L
Initial state	c_{S0} (A_c)	c_M	0	0
Equilibrium	c (A_c')	0	c_M	$c_L + n(c_{S0} - c_S)$

This is attributed to the fact that the protonation of amine (–NH_2 and –NH) in M and –COO^- both form many strong microelectrostatic fields. The assembly of S ions on M occurs by electrostatic attraction. Like the one described above, this assembly obeys the Langmuir isotherm adsorption. Thus, the characterization of MS_N may be performed by measuring the variation of the SL_n aggregate. The reaction factors were calculated by the relations

$$\gamma = m = \frac{c_{S0} - c_S}{c_M} = \frac{c_{S0} - c_S}{c_{S0}}\frac{c_{S0}}{c_M} = \eta\frac{c_{S0}}{c_M}, \quad (6)$$

$$c_S = c_{SL} = (1 - \eta)c_{S0}, \quad (7)$$

where

$$\eta = (A_c - A_c')/A_c = \Delta A_c/A_c. \quad (8)$$

A substitution reaction occurs in the above reaction (Fig. 1, *3*):

$$N\mathrm{SL}_n + \mathrm{M} = \mathrm{MS}_N + nN\mathrm{L},$$

when a protein of the (M) sort is added. This can be attributed to the fact that the protonation of both many aminos

(–NH_2 and –NH) in M and many –COO^- results in many microelectrostatic fields. This causes the assembly of S ions because of the electrostatic force. Similarly, the assembly of S on M also obeys the Langmuir isotherm adsorption. Thus, the characterization of MS_N can be done by analyzing the variations of SL_n and L.

EXPERIMENTAL

Materials

Absorption spectra were recorded on a Lambda-25 UV/VIS spectrometer (Perkin Elmer) with a 1-cm cell, and the individual absorbance was measured on a Model 722 spectrophotometer (Shanghai 3rd Analytical Instruments). The Drop Shape Analysis System

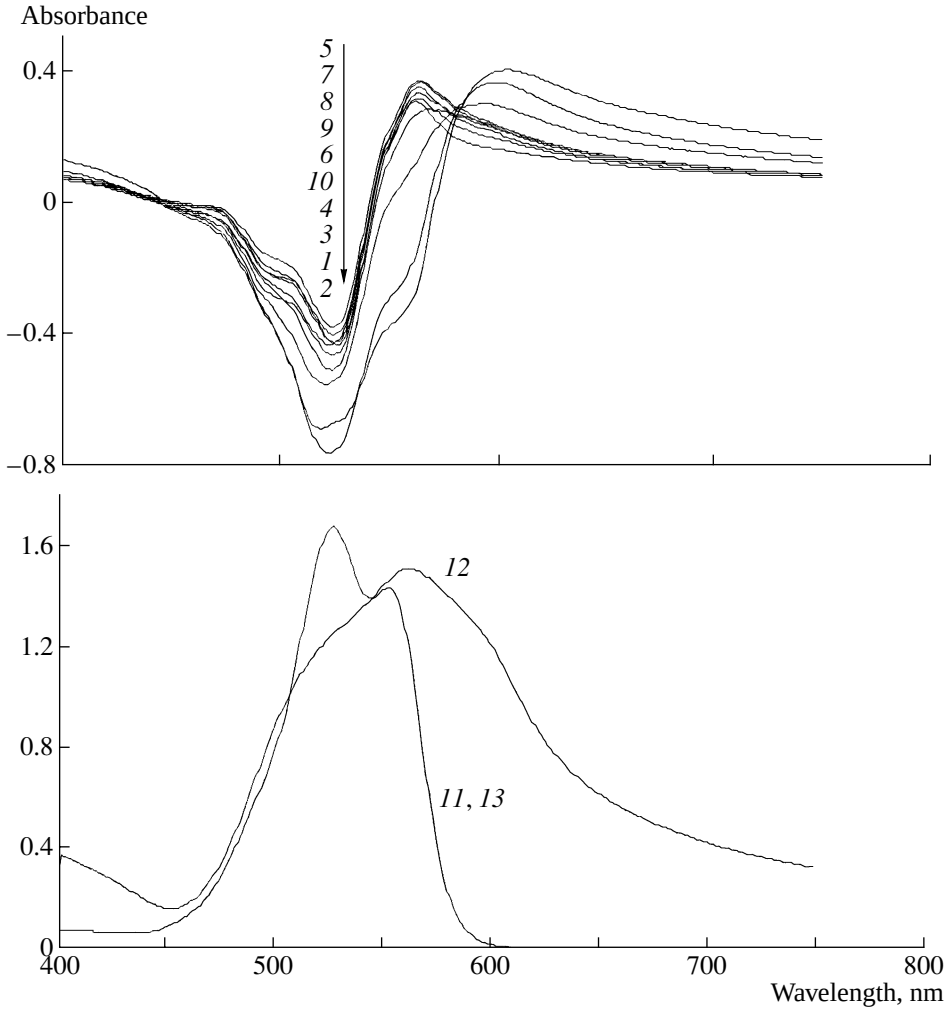


Fig. 2. Absorption spectra of PRB, PRB–SDBS, and RE–PRB–SDBS solutions: from curves *1–10*: at pH from 1.73, 2.81, 3.85, 4.88, 5.30, 5.81, 6.08, 7.00, 8.27, and 9.93, where the solutions contained 0.040 mmol/l SDBS and 0.040 mmol/l PRB, all measured against reagent blanks without SDBS; (*11*) 0.040 mmol/l PRB; (*12*) 0.040 mmol/l PRB + 0.100 mmol/l SDBS, and (*13*) 0.040 mmol/l PRB + 0.100 mmol/l SDBS + 0.100 mg/ml BSA, (*11, 12, 13*) all at pH 2.81 measured against water.

(DSA 10 MK 2, KRUSS GmbH, Germany) was used to measure the surface tension of solutions. The pH of solution was measured on a pHS-2C acidity meter (Leici Instruments, Shanghai). The temperature was adjusted and maintained constant in a Model 116R electric heated thermostat bath (Changjiang Test Instruments of Tongjiang, China).

Protein standard solutions were prepared by dissolving commercial bovine serum albumin (BSA) (Dongfeng Biological Technological, Shanghai), myoglobin (Mb) (Serva, Heidelberg, Germany), and ovalbumin (OVA) (Shanghai Chemical Reagents of the Chinese Medicine Group) in deionized water. The protein content (w, mg/ml) in the above solutions was determined and calculated using the relationship $w = 1.45A_{280} - 0.74A_{260}$ [31] by measuring their absorbances (A_{260} and A_{280}) at 260 and 280 nm via UV spectrophotometry. The standard stock solution of SDBS (1.00 mmol/l) was prepared by dissolving sodium dodecylbenzyl sulfonate (SDBS) (Shanghai Chemical Reagents) in deionized water. PRB solution (1.00 mmol/l) was prepared by dissolving 0.5982 g of pyronin B (PRB, content 60%, Sigma Chemicals) in 1000 ml of deionized water. Britton–Robinson buffer solutions of between pH 1.73 and 9.93 were prepared to control the acidity of the interaction solution. 2.0 mol/l NaCl was used to adjust the ionic strength of the aqueous solutions. A masking reagent solution, 1% Na_2EDTA, was prepared for masking metals in the determination of proteins.

Methods

Aggregation of PRB on SDBS. In a 25 ml calibrated flask, 2.5 ml of buffer solution (pH 2.81) and a known volume of 1.00 mmol/l PRB were added to an appropriate working solution of 1.00 mmol/l SDBS. The mixture was diluted to 25 ml with deionized water

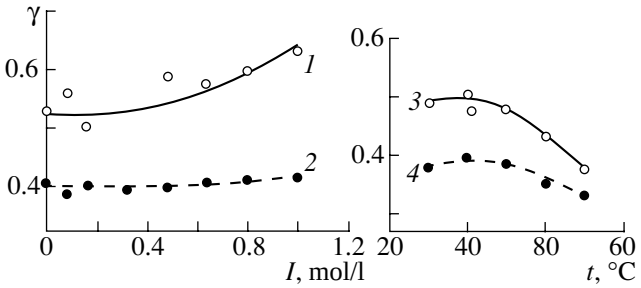


Fig. 3. Effect of ionic strength (*1*, *2*) and temperature (*3*, *4*) on the γ of PRB to SDBS: (*1*, *3*) 0.040 mmol/l SDBS + 0.040 mmol/l PRB and (*2*, *4*) 0.080 mmol/l SDBS + 0.040 mmol/l PRB.

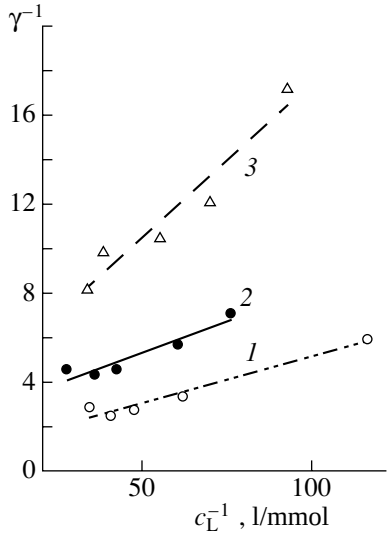


Fig. 4. Plots γ^{-1} vs. c_L^{-1} of the solutions containing 0.020 mmol/l SDBS and PRB between 0.0120 and 0.036 mmol/l: (*1*) 30°C (linear regression equation: $\gamma^{-1} = 0.0412c_L^{-1} + 1.01$), (*2*) 50°C ($\gamma^{-1} = 0.0571c_L^{-1} + 2.47$), and (*3*) 70°C ($\gamma^{-1} = 0.139c_L^{-1} + 3.55$).

and mixed thoroughly. After 5 min, absorbances were measured at 523 and 600 nm, respectively, against the blank (which was treated in the same way without SDBS); A_c, η, c_L, and γ were then calculated.

Aggregation of SDBS on proteins. In a 25-ml volumetric flask, 2.5 ml of pH 2.81 buffer solution was added to a known volume of 1.00 mmol/l SDBS. After mixing, 1.00 mmol/l PRB was added at 1.2 times SDBS molarity. This solution was diluted to 20 ml and mixed. After 5 min, a known amount of a protein solution was added. This solution was diluted to 25 ml and mixed well. After 10 min, the absorbances were measured at 523 and 600 nm against a blank treated in the same way but without any protein; ΔA_c, η, c_L, and γ were then calculated.

Two samples were prepared: sample **1** was a 0.1% milk powder liquid and sample **2** was a 5% children's drink. One-half milliliter of each sample was placed into 25-ml volumetric tubes. One milliliter of 1% EDTA was added and the subsequent operations were performed as in the above paragraph. Finally, the absorbance Δ*A* was measured at 523 nm against a reagent blank.

RESULTS AND DISCUSSION

Effect of pH and Spectral Analysis

The absorption spectra of the PRB and SDBS–PRB solutions with various pHs are shown in Fig. 2. By comparing curves *1–10*, we see that the reaction is most sensitive in acidic solution. This is attributed to the fact that PRB^{2-} was easy to form and was attracted strongly onto the SDBS. In contrast, only PRB^- was formed in neutral and basic solution, and its adsorption on S causes the blue shift of the peak wavelength and the decrease in the peak absorbance. Of the spectra shown, number *2* exhibits the maximum valley and the highest peak. In this work, a pH of 2.81 was selected. From curve *11*, the peak of the PRB is located at 527 nm. From curve *12*, the spectral peak of the PRB–SDBS aggregate is located at 563 nm. The spectral blue shift of the aggregate is only 36 nm. This is attributed to the fact that the electrostatic attraction is often much weaker than the chemical bond. The former causes only a little spectrum variation but the latter can bring an obvious spectral shift. However, from curve *2*, we observe that its peak and valley are located at 600 and 523 nm; two such wavelengths were used. In the SDBS–PRB solution, a great amount of BSA was added and the substitution of PRB was observed. Curve *13* shows the absorption spectrum of the substitution solution in the presence of a great amount of BSA. It is coincident with curve *11*, so the SDBS–PRB aggregate was destroyed by BSA and the PRB binding on SDBS was separated out. From curves *11* and *12*, the correction coefficients were calculated to be $\beta_{PRB} = 0.026$ and $\alpha_{SDBS-PRB} = 0.822$. The real absorbance of the SDBS–PRB aggregate was calculated by $A_c = 1.02(\Delta A - 0.026\Delta A')$.

Analysis of the Interaction of SDBS with PRB

A change was measured in the absorbance ratio A_{523}/A_{600} of solutions with various SDBS concentrations. The absorbance ratio remains minimal at a molar ratio of SDBS to PRB of between 2 and 10. No free PRB exists in the solutions containing 0.020 mmol/l PRB and 0.100 mmol/l SDBS. The ratio increases when the molar ratio of SDBS to PRB is over 10. This is attributed to the fact that a big colloid particle, PRB · $SDBS_n$ ($n \gg 1$), is formed; its spectrum shift is obvious. Here, the break point approach [21] was applied to the characterization of the noncovalent interaction, and the results showed that the break points of surface tension

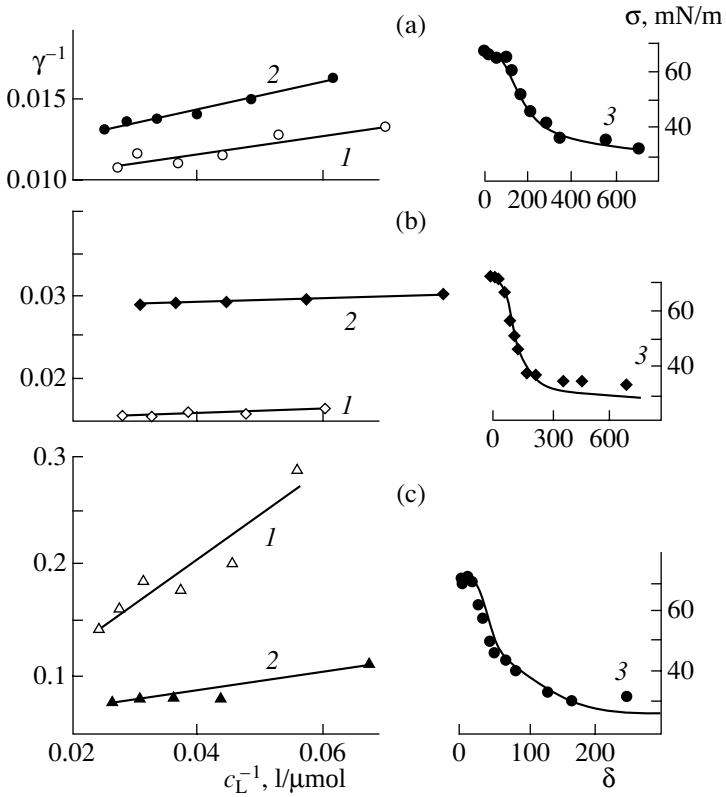


Fig. 5. Plots γ^{-1} vs. c_L^{-1} of the SDBS–protein solutions: (a) BSA, (b) OVA, and (c) Mb; (*1*) 30°C and (*2*) 50°C; (a) *1*, *2*, (b) *1*, *2*, and (c) *1*, *2* solutions containing 0.2365 mg of BSA, 0.2336 mg of OVA, and 0.2243 mg of Mb, respectively; all *1* and *2* containing SDBS between 0.024 and 0.060 mmol/l and PRB between 0.020 and 0.050 mmol/l; (*3*) surface tension variation of solutions containing 0.9477 mg of BSA (a), 0.9241 mg of OVA (b), and 0.9347 mg of Mb (c) (linear regression equations: (a) *1*: $\gamma^{-1} = 0.0612\,c_L^{-1} + 0.0091$, *2*: $\gamma^{-1} = 0.0885\,c_L^{-1} + 0.0108$; (b) *1*: $\gamma^{-1} = 0.0279\,c_L^{-1} + 0.0145$, *2*: $\gamma^{-1} = 0.0221\,c_L^{-1} + 0.0281$; (c) *1*: $\gamma^{-1} = 4.05\,c_L^{-1} + 0.0427$, *2*: $\gamma^{-1} = 0.880\,c_L^{-1} + 0.0517$).

variation of the solutions always appear at a molar ratio of SDBS to PRB of 1 : 1. Therefore, the maximum binding number *N* of PRB on SDBS was estimated to be 1; this will be examined below.

Effect of Ionic Strength and Temperature

In order to investigate the effect of the ionic strength of a solution on the aggregation of PRB, NaCl was added. From curves *1* and *2* in Fig. 3, the binding ratio of PRB to SDBS increases slowly with an increase in ionic strength. This is attributed to the fact that an increase in the ionic strength of a solution strengthens the ionization of PRB so that it is easily attracted onto SDBS.

From curves *3* and *4* in Fig. 3, we observe the effect of temperature on the binding ratio of PRB to SDBS. γ decreases with an increase in temperature, especially to over 50°C. This is attributed to the higher temperature causing the rapid desorption of PRB from the PRB–SDBS aggregate, which accords with the common nature of surface adsorption.

Characterization of the Aggregates

Varying SDBS molarity, the absorbance of the SDBS–PRB solutions was measured at three temperatures. Both the γ of PRB to SDBS and the c_L of PRB at equilibrium were calculated. Plots γ^{-1} vs. c_L^{-1} are shown in Fig. 4. We observe that all the curves are quite linear. Therefore, the aggregation of PRB on SDBS obeys the Langmuir isotherm adsorption. From the intercepts, the maximum binding number of the aggregate was calculated to be PRB : SDBS = 1 : 1 at 30°C, 2 : 5 at 50°C, and 2 : 7 at 70°C at pH 2.81. From this, we also know the desorption of PRB from its SDBS aggregate with an increase in temperature. From the slopes, the binding constant of the aggregate is calculated to be $K_{30°C} = 2.43 \times 10^4$, $K_{50°C} = 4.38 \times 10^4$, and $K_{70°C} = 2.50 \times 10^4$.

By simultaneously varying SDBS and PRB molarity at a constant ratio of 1 : 1.2, the absorbance of the SDBS–PRB–protein solutions was measured at 30 and 50°C (temperatures of more than 60°C cause the destruction of protein structure). Both the γ of SDBS to protein and the c_L of SDBS at equilibrium were calculated. Plots of γ^{-1} vs. c_L^{-1} are shown in Fig. 5. The aggregations of SDBS on BSA, OVA, and Mb all obey the Langmuir isotherm adsorption as well. From the intercepts, the maximal binding number (N) of the aggregate was calculated to be SDBS : BSA = 110 : 1 at 30°C and 93 : 1 at 50°C, SDBS : OVA = 69 : 1 at 30°C and 36 : 1 at 50°C, and SDBS : Mb = 23 : 1 at 30°C and 19 : 1 at 50°C. As in the previous paragraph, the maximum assembly number decreases with an increase in temperature. From the line slopes, the binding constants of the SDBS–protein aggregates are calculated to be $K_{\text{SDBS–BSA, 30°C}} = 6.73 \times 10^6$, $K_{\text{SDBS–BSA, 50°C}} = 8.19 \times 10^6$, $K_{\text{SDBS–OVA, 30°C}} = 1.92 \times 10^6$, $K_{\text{SDBS–OVA, 50°C}} = 0.79 \times 10^6$, $K_{\text{SDBS–Mb, 30°C}} = 9.48 \times 10^7$, and $K_{\text{SDBS–Mb, 50°C}} = 1.70 \times 10^7$.

In addition, from curves *3* in Fig. 5, their break points appear at 100 (Fig. 5a), 60 (Fig. 5b), and 20 (Fig. 5c), the ratios of SDBS to BSA, OVA, and Mb, respectively. The result is similar to the determinations above. The above maximum assembly number of SDBS on proteins is therefore accurate.

Application

The adsorption of SDBS on proteins with PRB as a spectral probe at pH 2.81 was applied to the quantitative detection of protein in the presence of EDTA. The standard series of various protein solutions was prepared and measured at 523 nm, and the regression equations were as follow:

BSA:

$$\Delta A = -0.4223x^2 + 0.6453x - 0.0026 \quad (R = 0.9973);$$

OVA:

$$\Delta A = -0.2565x^2 + 0.4095x + 0.0048 \quad (R = 0.9961);$$

and Mb:

$$\Delta A = -0.2628x^2 + 0.4805x - 0.0061 \quad (R = 0.9956)\ (x, \text{mg}).$$

Not all of the quadratic equations obeyed the Lambert–Beer law. Six replicated determinations of 0.243 mg of OVA were carried out, and the mean was 0.243 ± 0.006 mg.

By adding 1% EDTA to protein samples, the influence of foreign substances including ions, organic compounds, and other surfactants on the determination of BSA (as representative) was tested at pH 2.81. None of the following substances affected the direct determination of 0.300 mg of BSA (with a less than 10% error): 1 mg of Cl^-, SO_4^{2-}, Ac^-, 0.5 mg of Triton X-100, SDS, glucose, amino acid, Ca(II), Mg(II); 0.2 mg of DNA, RNA, F^-; 0.1 mg of Mn(II), Ni(II), Zn(II), Pb(II); 0.05 mg of Cu(II), Co(II), Cd(II), Fe(III), and Hg(II).

Two samples, milk powder (sample 1) and a children's drink (sample 2) were analyzed. The protein contents found in the samples accorded with the index mark given on the containers. The recovery of protein was between 90.0 and 113%, while the RSDs were less than 4.2%.

CONCLUSIONS

The investigation of the interactions of PRB with SDBS and SDBS with proteins supports the Langmuir monolayer aggregation of small molecules on macromolecules. While the MSASC technique does not have a higher sensitivity than other methods, it may yield satisfactory precision and accuracy and offer the additional benefits of simplicity and versatility. The application of the MSASC technique and the break point approach to the characterization of a macromolecular assembly product is different from the traditional methods, e.g., Scatchard [12], molar ratios, continuous variations, and equilibrium movements [32–34], since it solves the influence problem of a spectral probe background. It will play an important role in studying the interaction between macromolecules.

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