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Investigation on Molecular Non-covalent Interaction in the Sodium Dodecyl Benzene Sulfonate-polychrome Blue B-protein Replacement Reaction

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The molecular non-covalent interaction often originates from the electrostatic attraction and accords with the Langmuir isothermal adsorption. The sodium dodecyl benzene sulfonate (SDBS)-polychrome blue B (PCB)-protein [bovine serum albumin (BSA), ovalbumin (OVA) and myoglobin (MB)] ternary reaction has been investigated at pH 3.88. Protein to replace PCB from the PCB-SDBS binding product was used to characterize the assembly of an invisible-spectral compound, SDBS, on proteins by measuring the variation of PCB light-absorption by the micro-surface adsorption-spectral correction (MSASC) technique. The effect of ionic strength and temperature on the aggregation was studied. Results showed that the aggregates $SDBS_{92}$•BSA, $SDBS_{58}$•OVA and $SDBS_{15}$•MB at 30 ℃ and $SDBS_{83}$•BSA, $SDBS_{39}$•OVA and $SDBS_{10}$•MB at 50 ℃ are formed.

Keywords MSASC technique, non-covalent interaction, sodium dodecyl benzene sulfonate, polychrome blue B, protein, invisible-spectral molecule, replacement reaction

Introduction

Supramolecular assembly is always an important research direction for chemists and biochemists.[1-5] It is very helpful for us to accurately analyze the physico-chemical action and process occurring inside biological cell, for example, function of a drug, damage of a toxicant, detection of oncogene and so on. Understanding the chemical, physical, biological and pharmacological activity of a complex system requires knowledge of the state of molecular aggregation of the system components. In fact, the molecular interaction is very complicated. A product may be formed by covalent bond, coordinate bond and non-covalent bond, *e.g*., electrostatic attraction, hydrogen bond, van der Waals force, hydrophobic bond, insertion, winding of long-chain molecules. Usually, the co-action of a number of kinds of them exists in aqueous medium of cell. The electrostatic attraction is always a source force to attract the oppositely charged or polar molecules to junction then to induce the co-action of the other non-covalent bonds. The non-covalent interaction is often weaker than a covalent bond in a diluted medium so it is destroyed easily by a high temperature or a high ionic strength. Because it accords with the Langmuir isothermal adsorption, the non-covalent interaction could be regarded as a micro-surface adsorption reaction in only one monolayer in a diluted solution. Molecular spectrometry was extensively used[6-9] in study of biomacromolecular assembly and Pesavento,[10] Scatchard models[11, 12] and the micro-surface adsorption-spectral correction (MSASC)[13-15] are applied to characterization of the assembly. To characterize the interaction of an invisible spectral (IVS) molecule, *e.g*., drug, surfactant, pesticide and toxicant with biomacromolecule, this paper established a fit approach which described as follows: at first, a positively charged dye reacted with an IVS compound to form a product by non-covalent interaction and then a kind of protein was added into the product solution to replace the dye and to form the IVS compound-protein assembly product. Thus, the assembly product was characterized by measuring the variation of the dye light-absorption. Because the molecular non-covalent interaction often results in a little shift of the absorption spectra of solution, the MSASC technique must be applied to determination of the dye light-absorption in place of ordinary spectrophotometry. The sodium dodecyl benzene sulfonate (SDBS)-polychrome blue B (PCB)-protein [bovine serum albumin (BSA), ovalbumin (OVA) and myoglobin (MB)] ternary reaction has been investigated. PCB formed monovalent cation in aqueous solution and then was adsorbed on SDBS. Similarly, the protonation of amino groups (NH_2) on protein chain made itself carry a number of positive charges in acidic solution and then SDBS was attracted. The break point

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approach[16] was used as an auxiliary approach to characterize the assembly product. Results showed that the aggregation of PCB on SDBS and the assembly of SDBS on proteins obeyed the Langmuir isothermal adsorption. This approach is helpful for us to study the molecular interaction in cell in another way.

Experimental

Principle

In Figure 1, charged surfactant (S) (**1**) can attract oppositely charged stain probe (L) (**2**) and also the aggregation of L on S obeys the Langmuir isotherm adsorption.[17] Early, some synergism mechanisms,[18-22] *e.g.*, solubilization, stabilization and sensitivity enhancement were established and applied in, *e.g.*, synergism perturbation, hydrogen bond formation, micelle catalysis and others. On the basis of these models, the microsurface adsorption mechanism has been developed. It units both the micelle catalysis and electrostatic field aggregate theories and also improves them. Its combination with spectral correction method[23-25] named MSASC technique provided a very useful experimental strategy for study on the physicochemical interaction among molecules.

In Figure 1, it is seen that the stain probe (L) can be attracted and bound on an oppositely charged surfactant (S) (**1**) by electrostatic attraction, hydrogen bond, van der Waals force, hydrophobic bond and winding of long-chain molecules to form the product SL. The aggregation obeyed the Langmuir isotherm adsorption.[17] The SL solution equilibrium occurs as follows (m.s. means microsurface phase):

	nL + S (m.s.)	→	SL_n (m.s.)
Initial state	C_{L_0} (A_0, A_0') C_{S_0}		0
Equilibrium	C_L		C_{S_0} (A_c)

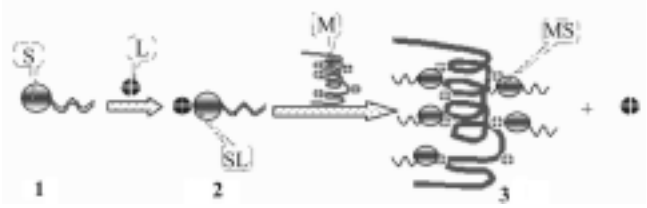

Figure 1 The aggregation of spectral probe (L) on surfactant (S) (**1**, **2**), substitution of L and assembly of S on biomacromolecule (M) (**3**).

The Langmuir isotherm equation is expressed as:

$$\frac{1}{\gamma}=\frac{1}{n}+\frac{1}{KnC_L} \tag{1}$$

where K is the equilibrium constant, C_L the molarity of the excess L, and γ the molar ratio of the effective L adsorbed to S. With increase in L concentration, γ approaches a maximal binding number, n. Both n and K may be calculated by plotting line γ^{-1} vs. C_S^{-1}. Both C_L and γ the same as n were calculated by the spectral correction equations:

$$\gamma = n = \eta \times \frac{C_{L_0}}{C_{S_0}} \tag{2}$$

$$C_L = (1-\eta)C_{L_0} \tag{3}$$

$$\eta = \frac{A_c - \Delta A}{A_0} \tag{4}$$

where both C_{S_0} and C_{L_0} are the initial molarities of S and L and η indicates the effective fraction of L. A_c, A_0 and ΔA are the real absorbance of the S-L product, the absorbance of L against water and that of the S-L solution against a reagent blank, respectively measured at the peak-absorption wavelength λ_2. A_c was calculated by means of:

$$A_c = \frac{\Delta A - \beta \Delta A'}{1-\alpha\beta} \tag{5}$$

where $\Delta A'$ indicates the absorbance of the S-L solution measured at the valley-absorption wavelength λ_1. Both α and β are the correction constants.

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

	SL_n + mM (m.s.)	→	MS_m (m.s.) + nL
Initial state	C_{S_0} (A_c) C_M		0
Equilibrium	C_S (A_c')		C_M $C_L + n(C_{S_0} - C_S)$

This was attributed to the protonation of amino groups (NH_2) in M. The assembly of S happens on M by the electrostatic attraction, hydrogen bond, van der Waals force, hydrophobic bond, insertion and winding of long-chain molecules. The assembly obeyed the Langmuir isotherm adsorption, too. Thus, the characterization of $MS_{N'}$ (N': maximal binding number) was made by measuring the variation of SL_n aggregate. The factors were calculated by the relations:

$$\gamma = m = \frac{C_{S_0} - C_S}{C_M} = \frac{C_{S_0} - C_S}{C_{S_0}} \times \frac{C_{S_0}}{C_M} = \eta \times \frac{C_{S_0}}{C_M} \tag{6}$$

$$C_S = C_{SL} = (1-\eta)C_{S_0} \tag{7}$$

where

$$\eta = \frac{A_c - A_c'}{A_c} = \frac{\Delta A_c}{A_c} \tag{8}$$

Materials and methods

Absorption spectra were recorded on a TU1901 spectrophotometer (P General, Beijing) and the individual absorbance was measured on a Model 722 spectrophotometer (Shanghai 3rd Analytical Instruments). The drop shape analysis System (DSA 10 MK 2, KRUSS GmbH Germany) was used to measure the surface tension of solutions. pH of solution was measured on a pHS-2C acidity meter (Leici Instruments, Shanghai). The temperature was adjusted and remained constant in an electronic heated thermostat bath, Model 116R (Changjiang Test Instruments of Tongjiang, China).

Protein standard solutions were prepared by dissolving the commercial bovine serum albumin (BSA) (Dongfeng Biological Technological, Shanghai), myoglobin (MB) (Serva, Heidelberg, Germany) and ovalbumin (OVA) (Shanghai Chemical Reagents of Chinese Medicine Group) in deionized water. The protein content (w, mg/mL) in the above solutions was determined and calculated by the relation: w (mg/mL)$=1.45A_{280\ nm}-0.74A_{260\ nm}$[26] by measuring their absorbances ($A_{260\ nm}$ and $A_{280\ nm}$) at 260 and 280 nm by UV spectrophotometry. The standard stock solution of SDBS (1.00 mmol/L) was prepared by dissolving sodium dodecyl benzene sulfonate (A.R., Hongguang Chemical Reagents of Yixing, China) in deionized water. PCB solution (0.500 mmol/L) was prepared by dissolving 0.2420 g of polychrome blue B ($C_{16}H_{18}N_3S\bullet Cl$, Sigma Chemicals) in 500 mL of deionized water. The Britton-Robinson buffer solutions between pH 1.73 and 9.93 were prepared to adjust the acidity of the solutions and 2.0 mol/L NaCl was used to adjust the ionic strength of the solutions. The masking reagent solution, 5% Na_2EDTA was prepared to mask metals in analysis of protein samples.

Experimental procedures

Aggregation of PCB on SDBS Into a 25 mL of calibrated flask were added an appropriate working solution of 1.00 mmol/L SDBS, 2.5 mL of buffer solution (pH 3.88) and a known volume of 0.500 mmol/L PCB. The mixture was diluted to 25 mL with deionized water and mixed thoroughly. After 5 min, the absorbances were measured at 488 and 600 nm, respectively against the blank treated in the same way without SDBS and then A_c, η, C_L and γ were calculated by Eqs. (2)—(5).

Aggregation of SDBS in proteins Into a 25 mL of volumetric flask, 2.5 mL of pH 3.88 buffer solution and a known volume of 1.00 mmol/L SDBS were added. After mixing, a known volume of 0.500 mmol/L PCB was added to keep a molarity as more as 1.2 times that of SDBS. The solution was diluted to about 20 mL and mixed. After 5 min, a known amount of a protein solution was added and then diluted to 25 mL. The resultant solution was mixed well. After 10 min, the absorbances were measured at 488 and 600 nm against the reagent blank treated in the same way without any protein and then ΔA_c, η, C_S and γ were calculated by Eqs. (5)—(8).

Determination of protein in samples Two sample solutions were prepared. Sample 1 was 0.1% milk powder liquid and sample 2 was 5% children drink. Into a 25 mL of volumetric flask, 2.5 mL of pH=3.88 buffer solution, 1.0 mL of 5% Na_2EDTA, 1.00 mL of 1.00 mmol/L SDBS and 4.00 mL of 0.500 mmol/L PCB were added. The mixture was diluted to 20 mL and mixed well. After 10 min, 0.50 mL of a sample solution was placed into the flask and the solution was diluted to 25 mL. After 10 min, the absorbance (ΔA) of the solution was measured at 600 nm against a reagent blank.

Results and discussion

Effect of pH and spectral analysis

The absorption spectra of the PCB and SDBS-PCB solutions with pH are shown in Figure 2. By comparing curves 1—9, it can be known that the reaction is the most sensitive in acidic solution. It is attributed to the fact that PCB^+ was formed easily because of the protonation of NH_2. On the contrary from curves 8 and 9, PCB cation will not be formed in basic medium. Among all of them, spectrum 2 gives the deepest valley and a highest peak. In this work, pH=3.88 was selected. The peak of PCB is located at 600 nm in curve 10 and that of the PCB-SDBS aggregate is located at 630 nm in curve 11. The spectral red shift of the aggregate is only 30 nm. This is just attributed to the weak non-covalent bond interaction in a diluted solution. In curve 2, the peak and valley of the SDBS-PCB solution against PCB

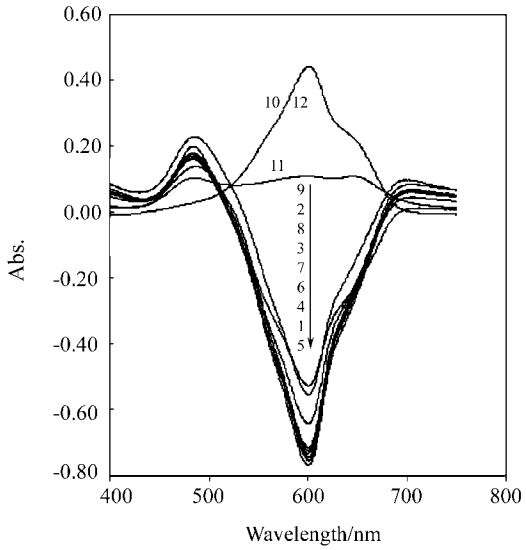

Figure 2 Absorption spectra of PCB, PCB-SDBS and PCB-SDBS-BSA. Solutions: from curves 1 to 9, at pH from 1.73, 3.85, 4.88, 5.30, 5.81, 6.08, 7.00, 8.27 and 9.93, where the solutions contained 0.040 mmol/L SDBS and 0.020 mmol/L PCB, all measured against the reagent blanks without SDBS. Curve 10, 0.020 mmol/L PCB; Curve 11, 0.020 mmol/L PCB plus 0.100 mmol/L SDBS; Curve 12, 0.020 mmol/L PCB plus 0.100 mmol/L SDBS plus 100 mg/L BSA. Curves 10, 11 and 12 all at pH 3.88 measured against water.

are located at 488 and 600 nm, so such two wavelengths were used. In an SDBS-PCB solution, plenty of BSA was added and the replacement of PCB from its SDBS aggregate was observed from the coincidence of curve 10 with curve 12. Curve 12 represents the absorption spectrum of the replacement solution where the PCB was replaced completely by BSA to form the ADBS-BSA aggregate. From curves 10 and 11, the correction coefficients were calculated to be $\beta_{PCB}=0.090$ and $\alpha_{SDBS\text{-}PCB}=0.672$. The real absorbance of the SDBS-PCB aggregate at 488 nm was calculated by $A_c=1.06\times(\Delta A-0.090\Delta A')$.

Auxiliary characterization of the break point aproach to interaction of SDBS with PCB and prteins

Curve A in Figure 3 indicates the change of the absorbance ratio, $A_{488\ nm}/A_{600\ nm}$ of the solutions with SDBS. The absorbance ratio approaches to a minimum when SDBS is more than 0.04 mmol/L. Therefore, no free PCB exists in the solutions where the molar ratio of SDBS to PCB is more than 1. The break point (P) of curve A is located at about 0.02 mmol/L SDBS, so the maximal binding number of PCB on SDBS was estimated to be 2. With the same method, from the variation of the surface tension of the SDBS-proteins solutions shown in Figure 3(B), their break points (P1, P2 and P3) were found easily to locate at P1≈72 (BSA), P2≈60 (OVA) and P3≈18 (MB). The binding numbers above will be still examined in the latter experiments.

Effect of ionic strength and temperature on the SDBS-PCB aggregation

In order to investigate the effect of ionic strength on the aggregation of PCB on SDBS, 2.0 mol/L NaCl was added into the SDBS-PCB solution. It was found that the binding ratio of PCB to SDBS decreased by about 15% per increasing 1 mol/L of ionic strength. This was attributed to the fact that much more Na^+ than PCB would enclose SDBS to oppose the binding of PCB. The effect of temperature has shown that γ of PCB to SDBS decreased by about 10%—15% per increasing 10 ℃. This was attributed to the fact that a higher temperature would often cause a rapid desorption of PCB from its SDBS aggregate.

Characterization of the SDBS-PCB aggregation

By varying SDBS molar concentration, the absorbance of the SDBS-PCB solutions was measured at three temperatures. γ of PCB to SDBS and C_L of PCB at equilibrium were calculated and plots γ^{-1} vs. C_L^{-1} are shown in Figure 4. All curves have good linearity. Consequently, the aggregation of PCB on SDBS accords with the Langmuir isotherm adsorption. From the intercepts, the maximal binding number N of PCB was calculated to be from $n=2$ at 30 ℃ to 1.5 at 70 ℃. The value at 30 ℃ is consistent with that above obtained by the break point approach. Therefore in the SDBS-PCB solution at pH 3.88, only PCB_2SDBS at 30 ℃, both about 50% PCB_2SDBS and about 50% PCB•SDBS at 50 ℃ and both about 30% PCB_2SDBS and about 70% PCB•SDBS at 70 ℃ were formed. The transition of the PCB-SDBS aggregate with temperature is sketched in Figure 4, too. From the slope of each curve, the binding constant of the aggregate was calculated to be $K_{SDBS\text{-}PCB,\ 30\ ℃}=1.39\times10^5$ L/mol, $K_{SDBS\text{-}PCB,\ 50\ ℃}=1.22\times10^5$ L/mol and $K_{SDBS\text{-}PCB,\ 70\ ℃}=6.29\times10^4$ L/mol.

Replacement of PCB and characterization of the SDBS-protein aggregation

In order to investigate the substitution of PCB and the assembly of SDBS on protein, the solutions containing SDBS and PCB at constant molar ratio of 1∶2 were prepared. The solutions consisted of the colour compounds: PCB and SDBS•PCB. They were used as a new chromogenic reagent to investigate the protein

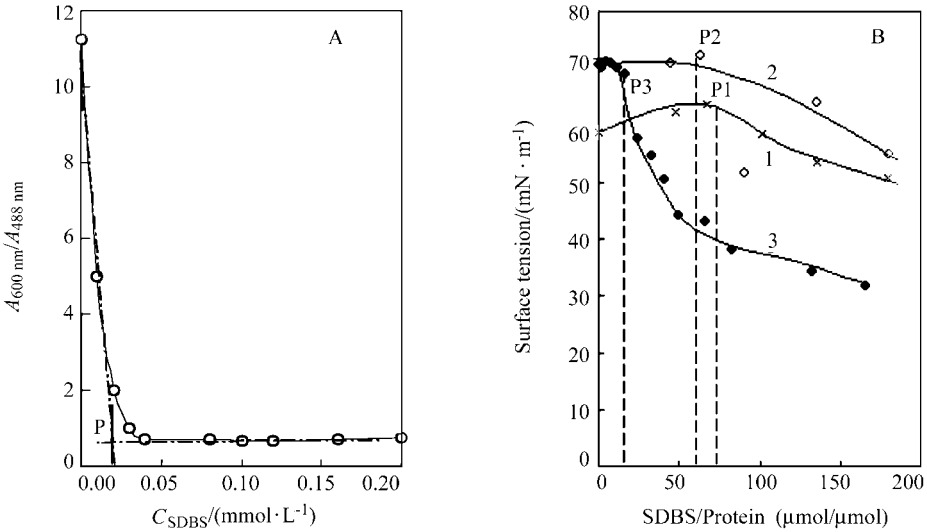

Figure 3 Application of the break point approach to auxiliary characterization of the binding number. (A) variation of A_{600nm}/A_{488nm} of the SDBS-PCB solutions containing 0.040 mmol/L PCB. (B) variation of the surface tension of the SDBS-protein solutions containing: 1, 38.0 mg/L BSA; 2, 37.0 mg/L OVA; 3, 37.4 mg/L MB, all at pH=3.88

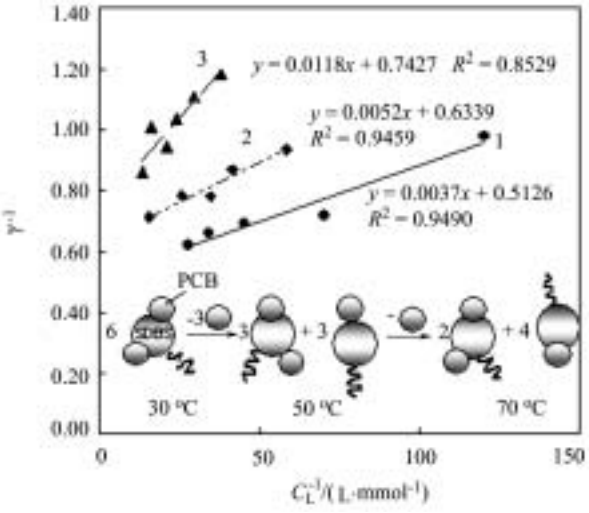

Figure 4 Plots γ^{-1} vs. C_L^{-1} for the solutions containing 0.020 mmol/L SDBS and PCB between 0.0120 and 0.0360 mmol/L. 1, 30 ℃; 2, 50 ℃; 3, 70 ℃; and sketch of transition of the SDBS-PCB aggregate with temperature.

assembly. By adding protein into the solution, it was observed that the solution approached to PCB color from SDBS•PCB product color. Therefore, the following replacement reaction occurred: SDBS•PCB + protein → SDBS•protein+PCB. The ternary solutions were measured at 30 and 50 ℃ according to the experimental procedure. γ of SDBS to protein and C_S of SDBS existed in form of SDBS·PCB at equilibrium were calculated by Eqs. (6)—(8). Plots γ^{-1} vs. C_S^{-1} are shown in Figure 5 and they are linear. Therefore, the aggregation of SDBS on BSA, OVA and MB obeyed the Langmuir isotherm adsorption.

From the intercepts, the maximal binding number of SDBS was calculated to be 92 in BSA at 30 ℃, and 83 at 50 ℃, 58 at 30 ℃ in OVA and 39 at 50 ℃, 15 at 30 ℃ in MB, and 10 at 50 ℃. The values at 30 ℃ are approximate to those above obtained by the break point approach. So, the effect of temperature on the assembly of small molecules on biomacromolecule is notable. The exact substitutions are expressed by the following reactions:

at 30 ℃

92 SDBS•PCB + BSA = $SDBS_{92}$•BSA + 92 PCB
58 SDBS•PCB + OVA = $SDBS_{58}$•OVA + 58 PCB
15 SDBS•PCB + MB = $SDBS_{15}$•MB + 15 PCB

at 50 ℃

83 SDBS•PCB + BSA = $SDBS_{83}$•BSA + 83 PCB
39 SDBS•PCB + OVA = $SDBS_{39}$•OVA + 39 PCB
10 SDBS•PCB + MB = $SDBS_{10}$•MB + 10 PCB

The maximal binding number decreased with increase in temperature because of the same reason as that discussed in the last paragraph. From variation of the binding number, the adsorption of SDBS on OVA is the most notable at high temperature. It was attributed to the fact that the third grade structure of OVA is the loosest among them to weaken the electrostatic attraction. From the linear slopes, the binding constants of the SDBS-protein aggregates was calculated to be

$K_{\text{SDBS-BSA, 30 ℃}} = 7.70\times10^3$, $K_{\text{SDBS-BSA, 50 ℃}} = 5.04\times10^5$, $K_{\text{SDBS-OVA, 30 ℃}} = 4.60\times10^3$, $K_{\text{SDBS-OVA, 50 ℃}} = 4.30\times10^4$, $K_{\text{SDBS-MB, 30 ℃}} = 5.38\times10^3$ and $K_{\text{SDBS-MB, 50 ℃}} = 1.17\times10^5$ L/mol.

By comparing K at 50 ℃ with that at 30 ℃, the attraction of SDBS in proteins at 50 ℃ is greater than that at 30 ℃. The binding constant $K_{\text{SDBS-PCB}}$ of the

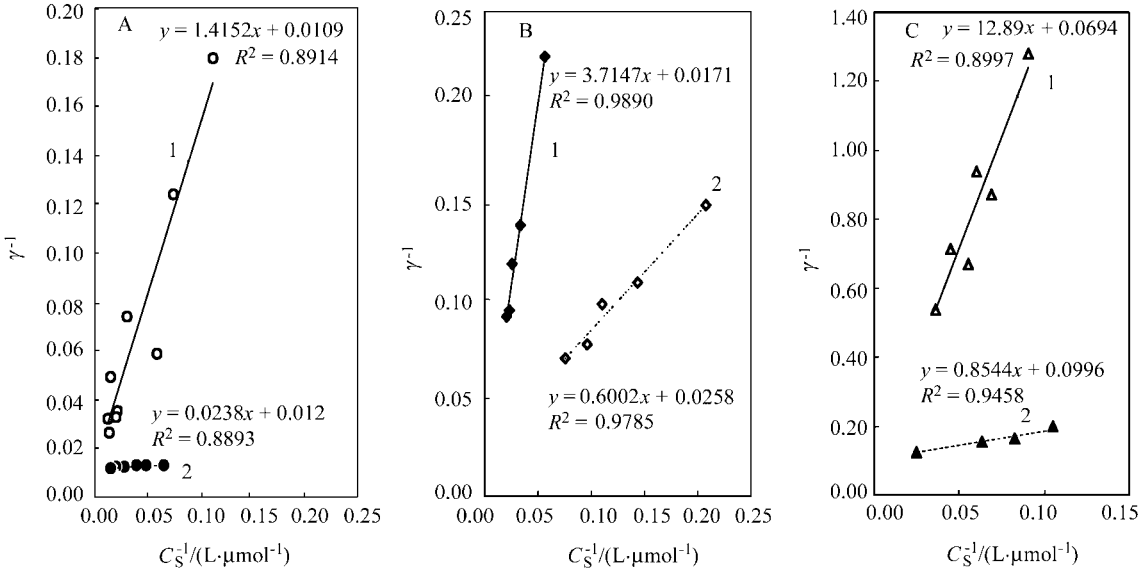

Figure 5 Plots of γ^{-1} vs. C_S^{-1} for the SDBS-protein solutions at pH=3.88. (A) BSA, (B) OVA and (C) MB. 1, 30 ℃; 2, 50 ℃. A-1 and A-2, B-1, and B-2, and C-1 and C-2 solutions contained 9.46 mg/L BSA, 9.34 mg/L OVA and 8.97 mg/L MB, respectively, which all initially contained SDBS between 0.024 and 0.060 mmol/L and PCB between 0.020 and 0.050 mmol/L.

SDBS-PCB at 50 ℃ is much less than that at 30 ℃ and PCB is desorbed easily from the SDBS aggregate at higher temperatures. At a high temperature, the protein molecule will unfold. The binding area of the SDBS long chain on protein will be increased by hydrophobic bonds and winding. The three dimensional effect will hinder the binding of the other SDBS on protein to result in decrease of the binding number. However, the increase of the binding area will make the binding energy (*K* as indicator) of SDBS on proteins high.

Application to detection of protein

The SDBS-PCB aggregation as the chromogenic reagent has been tried to apply to the quantitative analysis of protein in the presence of Na_2EDTA at pH=3.88. The standard series of various protein solutions were prepared and their absorbances were measured at 600 nm. The regression equations are followed: $y=0.0402x+0.1502$ in the range between 0 and 1.5 mg of BSA, $y=0.0741x+0.1336$ in the range between 0 and 7 mg of OVA and $y=0.09809x$ in the range between 0 and 0.4 mg of MB (*y*: absorbance measured at 600 nm and *x*: mg of protein). The second equation should be used in the determination of protein content in foods, *e.g*., milk powder, beverage.

By adding Na_2EDTA in protein samples to mask metal ions possibly existing in the solution, none of the following ions or compounds affected the direct determination of 1 mg of OVA (less than 10% error): 1 mg of vitamin C, Cl^- and SO_4^{2-}, 0.5 mg of glucose, tyrosine, glutamic acid, lysine and Ca(II), 0.2 mg of DNA and Fe(II) and 0.05 mg of Cu(II) , Pb(II), Zn(II), Mg(II), Co(II), Cd(II), Fe(III) and Hg(II).

Two samples, milk powder (1#) and children drink (2#) were analyzed with PCB-SDBS aggregate as chromogenic reagent. The protein contents (1#: 16.6% and 2#: 0.84%) in the samples accorded with the index mark signed (1#: 17% and 2#: less than 1%) on the containers. The recovery of protein was between 97.2% and 108% and the relative standard deviation less than 3.4%.

Conclusion

The investigation on the PCB-SDBS-protein ternary reaction indicated that the aggregation of PCB on SDBS and that of SDBS on proteins obeyed the Langmuir isothermal adsorption. The non-covalent interaction is easy to be destoryed by the concentrated eletrolyte and high temperature in a diluted medium. Also, the replacement reaction can be applied to the accurate determination of protein in foods. That proteins substitute the PCB bound on SDBS provided a useful way to characterize the interaction of an IVS compound with biomacromolecule. It will help to explain the molecular non-covalent interaction occurring in cell.

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