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Interaction of Tetra-iodophenolsulphonphthalein with Proteins

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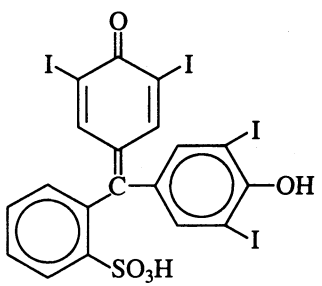
The formation of microelectrostatic fields in proteins is proposed and it causes the aggregation of stain. We have studied the interaction of tetra-iodophenolsulphonphthalein (TIPST) with two proteins: bovine serum albumin (BSA), ovalbumin (OVA) and lysoxyme (LYS). Results showed that the adsorption ratios of BSA, OVA and LYS are 10.5–8.00, 0.72–0 .64 and 0.81–0.64, respectively and their adsorption constants $K_{BSA\text{-}TIPST} = 3.34 \times 10^5$–$2.18 \times 10^5$, $K_{OVA\text{-}TIPST} = 1.50 \times 10^5$–$1.05 \times 10^5$ and $K_{LYS\text{-}TIPST} = 3.25 \times 10^4$–$5.34 \times 10^4$. and their absorptivities 2.48×10^5–2.29×10^5, 2.32×10^4–2.16×10^4 and 4.55×10^4–3.79×10^4 L mol^{-1} cm^{-1} at 628 nm.

Key Words: MPASC technique, Protein, Tetra-iodophenolsulphonphthalein, Microelectrostatic field, Langmuir aggregation.

INTRODUCTION

The interaction of proteins with small ions and molecules has become increasingly important to the chemist and the physician. Combinations of proteins with small anions and cations[1], sulfonamides[2], dyes[3], alkyl sulfates[4], fatty acids[5] and aromatic compounds[6] were earlier described. Today, more and more chemists and biochemists are interested in protein chemistry and its study becomes more and more active[7–9]. However, the interaction of a stain with protein has not been elucidated satisfactorily and earlier observations have not been explained clearly and reasonably, *e.g.*, the Pesavento equation[3]. The present work was undertaken in an attempt to clarify the general principles involved in protein-stain interaction. For this purpose, two kinds of proteins: bovine serum albumin (BSA), ovalbumin (OVA) and lysoxyme (LYS) and the stain tetra-iodophenolsulphonphthalein (TIPST) were selected in this study. The structure of TIPST is given below:



Tetra-iodophenolsulphonphthalein (TISPT)

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It may form anions in aqueous solution. We found that its aggregation is sensitive on protein (BSA, OVA and LYS) at pH 6.73 and such an aggregation obeys the Langmuir monolayer adsorption. Results showed that the adsorption ratio of BSA, OVA and LYS are 10.5–8.00, 0.72–0.64 and 0.81–0.64, respectively and their adsorption constants $K_{BSA\text{-}TIPST} = 3.34 \times 10^5$–$2.18 \times 10^5$, $K_{OVA\text{-}TIPST} = 1.50 \times 10^5$–$1.05 \times 10^5$ and $K_{LYS\text{-}TIPST} = 3.25 \times 10^4$–$5.34 \times 10^4$,

EXPERIMENTAL

Absorption spectra were recorded with a TU1901 spectrophotometer (PGeneral, Beijing) and an independent absorbance was measured on a Model 722 spectrophotometer (Shanghai 3rd Instruments). DDS-11A conductivity meter (Tianjin Second Analytical Instruments) was used to measure conductivity together with a DJS-1 conductivity immersion electrode (electrode constant 0.98) (Shanghai Tienkuang Devices) in the production of deionized water of lessthan 0.5 $(\mu\Omega\,cm)^{-1}$. The pH of the solution was measured with a 320-S pH-meter (Mettler-Toledo Instruments, Shanghai). The temperature was adjusted and remained constant in Model 116R electric heated thermostatic bath (Changjiang Test Instruments of Tongjiang, China).

Preparation of Solutions

Stock standard solutions of proteins were prepared by dissolving the commercial BSA (Shanghai Lizhu Dongfeng Biotechnology), OVA (Sigma) and LYS (Shanghai Lizhu Dongfeng Biotechnology) in deionized water. The protein content (w, mg/mL) in the above solutions was determined and calculated by the relation: $w = 1.45A_{280\,nm} - 0.74\,A_{260\,nm}$[13] by measuring their absorbances ($A_{260\,nm} - A_{280\,nm}$) at 260 and 280 nm by UV spectrophotometry. The solutions were diluted to 0.100 mg/mL for use. The TIPST solution, 0.2214 mmol/L, was prepared by dissolving 0.2000 g of tetra-iodophenolsulphonphthalein (TIPST, FW 857.97, dye content approx. 95%, B.D.H. Laboratory Chemicals, British Drug Houses) in 50 mL of N,N′-dimethylformamide (DMF, A.R., Wulian Chemicals, Shanghai) and then diluting to 1000 mL with deionized water. The Britton-Robinson buffer solutions between pH 4.88 and 9.48 were prepared to adjust the acidity of solutions. 2 mol/L NaCl was used to adjust the ionic strength of the aqueous solutions. All reagents were used without further purification.

Measurements

Into a 10 mL calibrated flask were added an appropriate working solution of proteins, 1.0 mL of Britton-Robinson buffer solution and appropriate TIPST solution. The mixture was then diluted to 10 mL with deionized water and mixed thoroughly. After 15 min, measurements were made against the blank treated in the same way without any protein.

Principal Equations and Calculation

In protein molecule, the protonization of the amino (—NH_2) in polar amino acids tend to form a positive electrostatic film and the negative carboxyl (—COO) to form a negative electrostatic film. Protein contains complex spatial structures, *e.g.*, winding, fold, coil and helix, and these cause cross of the double electrostatic

films to form many microelectrostatic fields[14]. They can attract ions till kinetic equilibrium (Fig. 1). The microelectrostatic field is so narrow that dye molecules are adsorbed in only a monolayer. Therefore, the aggregation is regarded as the

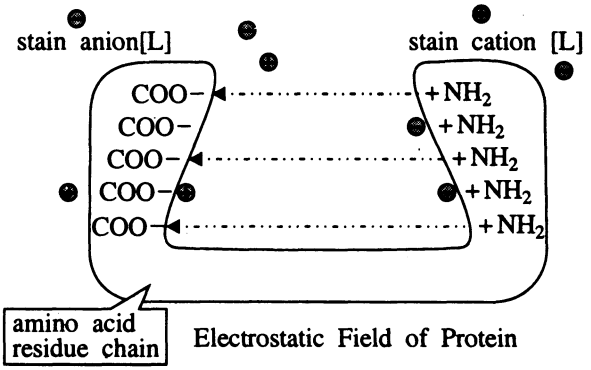

Fig. 1. Formation of microelectrostatic fields in protein and the adsorption process of stains (cations and anions) on microphase surface.

Langmuir isothermal adsorption[15]. The adsorption equilibrium is expressed as: L (aqueous phase, C_L) $\Leftrightarrow$ ML_N (protein phase, C_M) in L-M solution. The Langmuir adsorption formula is used:

$$\frac{1}{\gamma} = \frac{1}{N} + \frac{1}{KNC_L} \quad \ldots (1)$$

where K is the equilibrium constant and C_L the concentration of the excess L. N indicates the maximal adsorption ratio of L to M and γ the molar ratio of L adsorbed to M. K is calculated from equation 1. Both C_L and γ are calculated by means of Gao reports[16–19].

$$\gamma = \eta \times \frac{C_{L0}}{C_M} \quad \ldots (2)$$

$$C_L = (1 - \eta)C_{L0} \quad \ldots (3)$$

where

$$\eta = \frac{A_c - \Delta A}{A_0} \quad \ldots (4)$$

where both C_M and C_{L0} are the concentrations of the M and L added initially and η indicates the effective fraction of L. A_C, A_0 and ΔA are the real absorbances of the M-L product, the measurement absorbance of the reagent blank against water and that of the M-L solution against reagent blank directly measured at the peak wavelength λ_2. With increase of L concentration, γ will approach a maximum N. The A_c is calculated by the relation[20, 21]:

$$A_c = \frac{\Delta A - \beta \Delta A'}{1 - \alpha\beta} \quad \ldots (5)$$

where $\Delta A'$ indicates the absorbance of the M-L solution measured respectively at the valley absorption wavelength λ_1. Usually, α and β are the correction constants[22] and they are calculated by measuring directly ML_N and L solutions. In addition, the absorptivity (real $\varepsilon_r^{\lambda_2}$ not apparent $\varepsilon_a^{\lambda_2}$) of the adsorption product ML_N at λ_2 is also directly calculated by the following equation.

$$\varepsilon_r^{\lambda_2} = \frac{NA_c}{\delta\gamma C_M} \quad \ldots(6)$$

where δ is the cell thickness (cm) and the others have the same meanings as above.

RESULTS AND DISCUSSION

Effect of pH on Absorption Spectra

At various pHs, the adsorption of TIPST with protein (BSA as representative) was carried out. Their absorption spectra are shown in Fig. 2. By comparing each

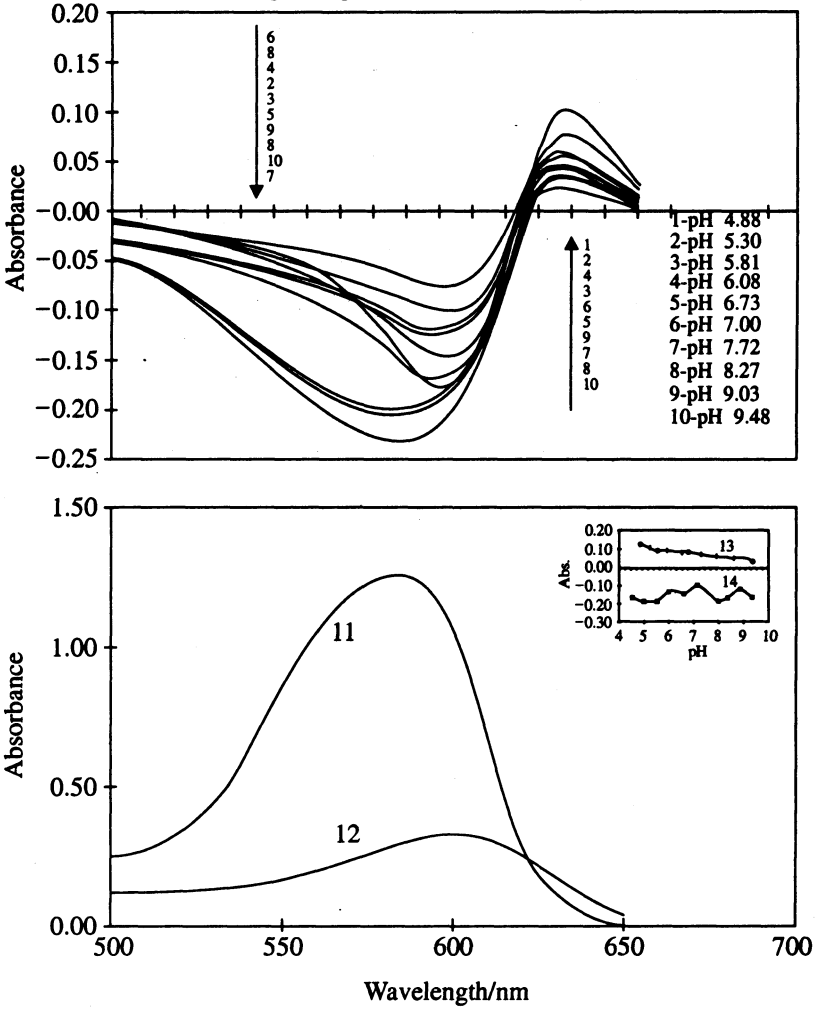

Fig. 2. Absorption spectra of TIPST (0.2214 μmol) and its BSA (0.40 mg) solutions: 1-pH 4.88; 2-pH 5.30; 3-pH 5.81; 4-pH 6.08; 5-pH 6.73; 6-pH 7.00; 7-pH 7.72; 8-pH 8.27; 9-pH 9.03; 10-pH 9.48. curves above all against reagent blank. 11-TIPST solution and 12- the solution initially containing 0.2214 μmol of TIPST and 15 mg of BSA, both them against water. 13- effect of pH on the absorbances measured at 628 nm and 14- same as 13 but at 592.5 nm, both these against reagent blank.

other, the pH 6.73 gives a sensitive aggregation of BSA with TIPST. This is attributed to the fact that TIPST may form enough anions to easily bind the proteins. In this work, pH 6.73 buffer solution was used. In this study, such a pH solution was selected. Curves 11 and 12 show the spectra of the TIPST and its BSA aggregate at pH 6.73, where the peak of TIPST is located at 585.5 nm and that of the BSA-TIPST aggregate at 606.5 nm. The spectral red shift of the aggregate is only 21 nm. Therefore, the excessive TIPST will interfere in the measurement of real absorbance of the aggregate. From the relative spectrum 3, we observe that its peak is located at 628 nm and the valley at 592.5 nm. Such two wavelengths were used in this work. From curves 11 and 12, the correction coefficients were calculated to be $\beta = 0.148$ and $\alpha = 1.48$. Because of high β, the spectral correction method was used in place of ordinary spectrophotometry and $A_c = 1.28(\Delta A - 0.148\Delta A')$.

Effect of Ionic Strength and Temperature

The influence of ionic strength on the binding ratio of TIPST to BSA is shown in Fig. 3. From curve 1, the adsorption ratio of TIPST to BSA becomes less in the presence of NaCl. This is attributed to the fact that the high concentration Cl anions may be bound on protein to occupy the electrostatic fields.

At various temperatures, the binding ratio of TIPST to BSA is shown in Fig. 3, too. From curve 2, we observe that the ratio decreases by 10% per increase of 10°C between 20 and 60°C. This is attributed to the fact that the desorption of TIPST occurs easily from protein at higher temperature. This accord with the nature of surface chemistry.

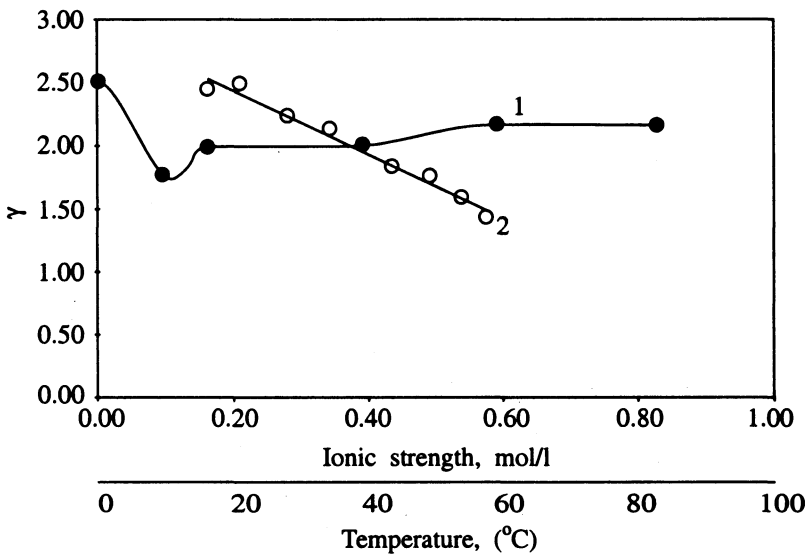

Fig. 3. Effect of ionic strength (1) and temperature (2) on the binding ratio of the BSA-TIPST aggregate in the solutions initially containing 0.2214 μmol of TIPST and 1.736 mg of BSA.

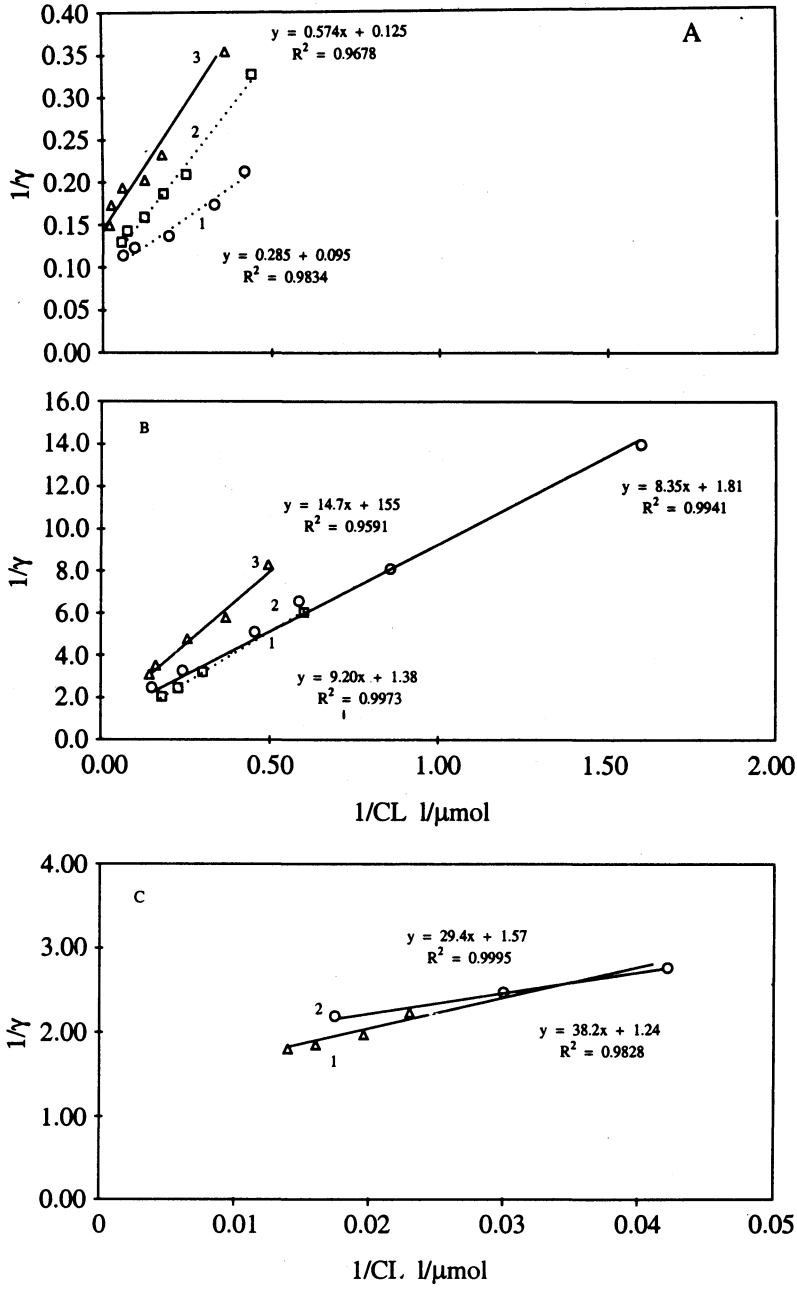

Fig. 4. Effect of addition of 0.2214 mM TIPST on the protein-TIPST aggregate (A) γ^{-1} *vs.* C_L^{-1} for the BSA (1.00 mg)-TIPST (between 0.0664 and 0.3321 μmol) solution; (B) same as (A) but for the OVA (3.00 mg)-TIPST (between 0.011 and 0.133 μmol) solution; (C) same as (A) but for the LYS (4.04 mg)-TIPST (between 0.0664 and 0.443 μmol) solution; (1) 20°C, (2) 40°C and (3) 60°C.

Determination of Property Constants of the Aggregates

By varying the addition of TIPST solution, the absorption of the various protein solutions with TIPST was measured and calculated the values of γ and C_L of each above solution. Their relationship is also shown in Fig. 4. All experimental points are linear at three temperatures (at 60°C, LYS-TIPST solution appears turbid.), so the aggregation of TIPST on BSA, OVA and LYS obeys the Langmuir isothermal adsorption. From the regressed expressions shown in Fig. 4, we calculate the adsorption ratio of TIPST to BSA, OVA and LYS and the binding constants of the aggregates and their molar absorptivities at 700.4 nm. The results are given in Table-1. In the determination of the adsorption ratio and adsorption constant, the spectral correction method had special advantage in operation and principle by contrast of classical methods such as molar ratio[23], etc.

TABLE-1
DETERMINATION OF THE AGGREGATES OF BSA, OVA AND LYS WITH TIPST AT pH 6.73

Interaction	Temp. (°C)	Maximal binding ratio	Binding constant, K	Molar absorptivity at 628 nm, L mol^{-1} cm^{-1}
BSA-TIPST	20	TIPST : BSA = 10.5 : 1	3.34×10^5	2.48×10^5
	40	TIPST : BSA = 9.4 : 1	2.22×10^5	2.32×10^5
	60	TIPST : BSA = 8.0 : 1	2.18×10^5	2.29×10^5
OVA-TIPST	20	TIPST : OVA = 0.72 : 1	1.50×10^5	2.32×10^4
	40	TIPST : OVA = 0.55 : 1	2.17×10^5	2.00×10^4
	60	TIPST : OVA = 0.64 : 1	1.05×10^5	2.16×10^4
LYS-TIPST	20	TIPST : LYS = 0.81 : 1	3.25×10^4	4.55×10^4
	40	TIPST : LYS = 0.64 : 1	5.34×10^4	3.79×10^4

Conclusion

The investigation to the aggregation of TIPST in proteins supports the Langmuir monolayer adsorption of stain on biomacromolecule. Though MPASC technique has not given higher sensitivity than other methods such as RLS[11], it meets precision and accuracy criteria and offers the additional benefits of simplicity and versatility. We understand that the classical method can still play an important role in macromolecular behaviour and interaction with small ions or stains.

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