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Spectrophotometric Study on the Interaction between Arsenazo M and Proteins

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Abstract: Arsenazo M could bind with bovine serum albumin to form a complex in Clark-Lube buffer at pH 2.3 and room temperature, which gives a maximum absorption peak at 625 nm with a red shift of 75 nm compared with that of Arsenazo M itself. The apparent molar absorptivity of the BSA-Arsenazo M complex is 3.21×10^5 $L\cdot mol^{-1}\cdot cm^{-1}$. The linear ranges for protein determination are wide (at least 0-100 μg/mL).

Keywords: Arsenazo M, serum proteins, spectrophotometry.

Dye binding protein assays are commonly used in biochemical and clinical laboratories, and the reaction mechanism is still under investigation[1-2]. In this paper, the interaction of Arsenazo M with serum proteins was studied.

Procedure

In most experiments, 1.20 mL of Arsenazo M (5.00×10^{-4} mol/L), 2.0 mL of C-L buffer solution, 3.0 mL alcohol and certain amount of protein standard solutions or samples were added to 10 mL volumetric flasks, then diluted to mark with water, and mixed thoroughly. After 5 min at room temperature, the absorption spectrum or absorbance of the solution was measured at 625 nm with 1 cm cell against the reagent blank.

Results and Discussion

Under optimum experimental conditions, Arsenazo M is red-purple with maximum absorption at 550 nm. When BSA was added into Arsenazo M solution, a purple-blue color was observed, which indicated the formation of the Arsenazo M-BSA complex with an absorption peak at 625 nm (other proteins are also the same), the peak wavelength showed a red shift of 75 nm. The absorbance of the complex at 625 nm is proportional to the amount of BSA.

The absorbance of Arsenazo M-protein is stable in acidic solution in the range of pH 1.6 ~ 3.0, 2.0 mL of C-L buffer (pH 2.3) was chosen to control the pH of solutions. The maximum binding number of Arsenazo M to BSA is 34 by molar ratio method.

The presence of glycerol and acetone lower the absorbance, but alcohol increases

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the absorbance and speeds up the reaction. When 3.0 mL alcohol was added, the absorbance raised 25% than without alcohol at the same temperature. The reason may be that the helix structure of protein is changed by the presence of alcohol. High extension of polypeptide chain is beneficial for binding of Arsenazo M with negative charge to $-NH_3^+$ group in proteins.

The effect of reaction time was tested at 15℃(room temperature)without alcohol, also at 15°C, 25°C, 35°C (water bath)with alcohol, respectively. The interaction between Arsenazo M and BSA occurs rapidly at the above three temperatures (about 5 min), but sensitivity is not the same. The higher the temperature is, the higher the sensitivity. We choose room temperature and 5 min for convenience and stability. The complex is stable for at least 2 h.

The effect of ionic strength on the reaction of Arsenazo M with BSA was tested with NaC1. In acid solution, the Arsenazo M has negative charge, and the proteins have positive charge, therefore the complexes are formed by electrostatic attraction. The absorbance of solution is reduced by shielding effect of ions, higher ionic strength resistes the complexation. Calibration curves of some proteins and effects of coexisting substances were studied. They are listed in **Table 1** and **Table 2**.

Table 1 Parameters for protein determination (n = 9)

Proteins	Linear regression equation (r /μg·mL^{-1})	Linear range (μg·mL^{-1})	r	e (L·mol^{-1}·cm^{-1})	S (μg·cm^{-2})
BSA	A=4.71×10^{-3} r -0.010	0~160	0.9996	3.21×10^5	0.21
γ-G	A=2.04×10^{-3} r -0.026	0~200	0.9995	3.67×10^5	0.48
HSA	A=3.94×10^{-3} r -0.024	0~100	0.9963	2.31×10^5	0.25
Hb	A=6.21×10^{-3} r -0.015	0~120	0.9983	4.16×10^5	0.16
Lyso	A=3.73×10^{-3} r -0.001	0~140	0.9996	5.37×10^4	0.26

Table 2 Effects of coexisting substances (c(BSA)=1.47×10^{-6}mol/L)

Substances	Leu	Ser	Glu	Lys	Cys	Tyr	Pro	Pb^{2+}	Ca^{2+}	Cu^{2+}	glucose	citric acid
r/(μg/mL)	30	30	30	30	30	30	30	2	2	2	100	50
Error(%)	-1.5	-2.0	-3.8	1.0	4.3	4.3	4.3	-4.2	-4.2	0.0	4.8	-1.4

Application on human serum assay

Transfer a suitable amount of sample solution to a 10 mL volumetric flask and determine total proteins by the procedure above and by classical method[3], respectively. The results are listed in **Table 3**. The recovery is satisfactory.

Table 3 Assay results of total proteins in human serum sample (n =3)

Method	Content (mg/mL)	Recovery(%)	RSD(%)
CBB G-250[3]	77.5		1.66
Arsenazo M	76.3	98.3	0.85

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