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Spectroscopic studies on the binding of bromocresol purple to different serum albumins and its bilirubin displacing action

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Abstract

The interaction between bromocresol purple (BCP) and bovine serum albumin (BSA)/porcine serum albumin (PSA) was investigated both in the absence and presence of bilirubin (BR) using absorption/absorption difference spectroscopy. A significant red shift in the absorption maxima of BCP accompanied by a decrease in absorbance was indicative of BCP binding to albumin. The titration of BSA and PSA with BCP using absorption difference spectroscopy and analysis of results by Benesi–Hildebrand equation yielded the values of association constant, K as $9.9 \pm 0.9 \times 10^4$ L mol^{-1} and $4.1 \pm 0.3 \times 10^4$ L mol^{-1} for BSA and PSA, respectively. The differential extinction coefficient ($\Delta\varepsilon$) of 34,484 M^{-1} cm^{-1} at 615 nm and 41,870 M^{-1} cm^{-1} at 619 nm were estimated for BSA and PSA, respectively. Decrease in $(\Delta\text{Abs.})_{615\,\text{nm}}$ of BCP–BSA complex with the increase in ionic strength suggested the role of hydrophobic interactions in the binding phenomenon. A significant blue shift in the absorption maxima and change in $(\Delta\text{Abs.})_{\lambda_{max}}$ values of BR–albumin complexes upon addition of increasing concentrations of BCP revealed the BR displacing action of BCP on albumin molecule.

Keywords: Absorption difference spectra; Bilirubin displacement; Bovine serum albumin; Bromocresol purple; Porcine serum albumin

1. Introduction

Serum albumin is the most abundant protein in mammalian blood circulation and is characterized as the major transport protein due to its ability to bind a number of endogenous and exogenous compounds [1,2]. Binding of bilirubin (BR), an endogenous compound produced as a result of heme catabolism, to serum albumin is of physiological relevance as increase in free BR concentration in plasma may lead to the development of kernicterus in newborn infants [3,4]. Several anionic ligands including drugs and dyes also bind competitively at the same site on albumin where BR binds and displace BR from albumin [2,4–8]. A detailed knowledge about the binding mechanism of BR and other organic anions to albumin is required to get more insight about the BR binding site. These studies are important for the development of preventive measures against kernicterus [9,10]. However, the approach is hampered by several factors including the lack of a suitable animal model as different mammalian albumins have shown differences in the BR binding phenomenon including microenvironment of BR binding site [11–15]. Therefore, it becomes necessary to carefully consider the species differences. Binding studies of other competitive ligands to serum albumins will add towards checking the similarities in these albumins with reference to BR binding site.

Several dyes have been tested for their binding to albumin in order to develop protein assays [16], protein purification methods [17] and getting insights about a binding site [2,18]. Though binding of phenolsulfophthalein and sulfobromophthalein dyes to human serum albumin (HSA) and bovine serum albumin (BSA) along with their competition with BR have been shown [19,20], binding of bromocresol purple (BCP) to different mammalian albumins and its BR displacing action has not been studied so far. Recently, Kamat and Seetharamappa [21] have reported fluorescence

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and circular dichroism studies on the interaction of BCP with bovine serum albumin (BSA). Here, we present our data on BCP binding to two mammalian albumins, namely BSA and porcine serum albumin (PSA) and its BR displacing action.

2. Experimental

2.1. Materials

BR (lot 055K0919), BSA (lot 015K0591) and PSA (lot 084K7636) were purchased from Sigma Chemical Company, St. Louis MO, USA. BCP (lot 30797) was the product of United States Biochemical Corporation, Cleveland, OH, USA. All other chemicals were of analytical grade.

2.2. Analytical methods

Protein concentration was determined either spectrophotometrically using $E^{1\%}_{1\,cm}$ of 6.67 at 279 nm for BSA [22] or by the method of Lowry et al. [23] using BSA as the standard.

2.3. Preparation of solutions

BR stock solution was prepared by dissolving 3 mg of BR in 1 ml of 0.5 N sodium hydroxide solution containing 1 mM EDTA and the desired volume was made up to 50 ml with 5 mM sodium phosphate buffer, pH 8.0 containing 0.14 M NaCl (0.15PB8-NaCl). This solution was centrifuged at 5000 rpm for 10 min to remove insoluble ingredients, if any and stored in dark. BR concentration of the centrifuged stock solution was determined spectrophotometrically by taking absorbance of BR solution after diluting it 10 times with the same buffer at 440 nm and using a molar extinction coefficient of 47,500 [24]. The final concentration of the stock solution was 100 μM. The solution was prepared fresh and used within 2 h. All experiments involving BR were performed in dim light.

BCP stock solution (100 μM) was prepared by dissolving 5.62 mg of solid BCP in 100 ml of 0.15PB8-NaCl. This solution was used within a week.

Protein stock solutions (50 μM) were also prepared in 25 ml of 0.15PB8-NaCl by dissolving 83.75 mg of BSA/PSA in it.

2.4. Absorption/absorption difference spectroscopy

Spectral measurements (absorption spectra and absorption difference spectra) were made in the visible region on a Shimadzu double beam spectrophotometer, model UV-2450 using quartz cuvettes of 1 cm path length.

2.5. BCP–albumin binding studies

Absorption spectra of free BCP and BCP–albumin complexes were recorded in the wavelength range, 500–650 nm at different [BCP]/[albumin] molar ratios. Different molar ratios were obtained by keeping the albumin concentration constant (10 μM) while varying the BCP concentration. Increasing volumes of stock BCP solution (0.5, 1.0, 1.5 and 2.0 ml) were added to 1 ml of protein solution taken in four different tubes to get [BCP]/[albumin] molar ratio of 1:1, 2:1, 3:1 and 4:1, respectively. The total volume was made to 5.0 ml using 0.15PB8-NaCl. Albumin free BCP solutions were prepared by taking similar volumes (0.5, 1.0, 1.5, 2.0 ml) of stock BCP solution without albumin in a total volume of 5.0 ml.

The titration of albumin (BSA/PSA) with BCP was carried out by adding increasing volumes (0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45 and 0.5 ml) of stock BCP solution to 1 ml of protein solution (final concentration, 10 μM) taken in different tubes to obtain different [BCP]/[albumin] molar ratios in the range of 0.1–1.0. The final volume of incubation mixture in each tube was made to 5.0 ml with 0.15PB8-NaCl. Blanks were prepared in the same way except that buffer was used instead of protein solution. Absorption difference spectra were recorded in the wavelength range 500–650 nm. Values of positive absorbance difference at λ_{max}, $(\Delta Abs.)_{\lambda_{max}}$ at different BCP concentrations were used in the determination of binding constant of BCP to albumins. Reciprocal of $(\Delta Abs.)_{\lambda_{max}}$ was plotted against reciprocal of BCP concentration and analyzed according to the Benesi–Hildebrand equation as shown earlier [25]

$$\frac{1}{\Delta A} = \frac{1}{K\Delta\varepsilon[SA]}\frac{1}{[BCP]} + \frac{1}{\Delta\varepsilon[SA]} \quad (1)$$

where ΔA is the absorbance difference at λ_{max} at different concentrations of BCP varying from 1.0 to 10 μM, [SA] is the total concentration of either BSA or PSA and [BCP] is the total concentration of BCP. $\Delta\varepsilon$ is the differential extinction coefficient at λ_{max} for BCP binding to BSA or PSA and K is the association constant.

Effect of ionic strength on BCP–albumin interaction was studied by absorption difference spectroscopy in sodium phosphate buffer, pH 8.0. Ionic strength adjustments were made by adding desired amount of sodium chloride. Absorption difference spectra of 2:1 BCP–BSA/PSA mixture were recorded in the wavelength range, 500–650 nm at three different ionic strengths, *i.e.* 0.015, 0.15 and 1.0 M, respectively.

2.6. BR displacement studies

BCP-induced BR displacement from albumin was studied by absorption difference spectroscopy. Increasing volumes (0.4, 0.8, 1.2 and 1.6 ml) of stock BCP solution were added to a (1:1) BR–albumin mixture containing 0.8 ml of albumin and 0.4 ml of BR solutions (final concentration, 8 μM each) in different tubes to obtain different BCP–albumin molar ratios (1:1, 2:1, 3:1 and 4:1). The total volume of the incubation mixture was made to 5.0 ml with 0.15PB8-NaCl. Various controls/blanks were prepared in