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FULL PAPER

In Vitro Study of Interactions of Carboxamide Derivatives of Amino Acid with BSA: Ultrasonic Interferometer

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Abstract:

In this paper we account the interaction of the Carboxamide derivatives of amino acid viz 2-{[2-(cyclohexycarbamoyl) benzoyl] amino} propanoic acid (**2CMPA**), 2-Benzamido acetic acid 2-cyclohexyl carboxamide (**2BA2C**), 2-{[2-(cyclohexylcarbamoyl) benzoyl] amino}-3-methylbutanoic acid (**2CA3MBA**), 2-benzamido-4-methylpentanoic acid-2-cyclohexyl carboxamide (**2-BMCA**) and 2-{[2-(cyclohexycarbamoyl) benzoyl] amino}-4-(methylsulfanyl) butanoic acid (**2CA4MBA**) with protein Bovine serum albumin (BSA) using ultrasonic interferometer technique. Ultrasonic velocity for complex solution of different compounds of carboxamide derivatives of amino acid with BSA has been measured at their different composition using ultrasonic interferometer. Difference in the ultrasonic velocity at different compositions of complex is measure of binding of the compounds with BSA. Binding effect at various pH viz. 3, 4 and 5 shows that compounds bound to the BSA more significantly at acidic pH and association constant decreases with increase in pH value. Scatchard analysis gives the values of association constants (K_f) for all the compounds at pH 3, 4 and 5 respectively.

Keywords: ultrasonic interferometer; bovine serum albumin; carboxamide; association constant; scatchard analysis

1. Introduction

Binding of drug to plasma protein is one of the efficient biological characteristics of that drug. There are various proteins which show affinity for the drugs depending on their nature. There are various plasma protein such as Human serum albumin (HSA) and Bovine serum albumin Alpha acid glycoprotein (AGP) and Lipoprotein etc which shows affinity towards the drugs. These proteins perform various functions out of that drug binding and their transportation is an important one.

BSA is the moiety with large molecular weight (M_r = 66,500) contains 583 amino acids. As BSA is a major protein in blood, any change in level of BSA produces effect on transportation of drug. BSA is alkaline having 7-8 pH range [1] hence it shows the affinity for acidic drugs. There are various forces which are responsible for binding of drug to plasma protein viz. hydrogen bonding, vander wall forces, electrostatic attraction etc.

Binding study of various drugs with plasma proteins has been done such as the effect of binding on specific site of BSA for ciprofloxacin and captopril drugs in presence of specific site probe studied using equilibrium dialysis [2]. The protein-protein and protein-ligand interactions involved in retinol transport studied in plasma [3]. Interaction of drugs like i-bruprofen & naproxen shows successive binding to protein [4]. Effect of arsenic on binding of protein with warfarin and acetaminophenol observed [5]. Crystal structure analysis of binding of warfarin to BSA also studied [6]. NMR Spectroscopic approach reveals metabolic diversity of human blood plasma associated with protein drug interaction [7].

Effect of arsenic on binding of paracetamol

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with BSA was studied using equilibrium dialysis method [8]. Thin layer chromatography technique used for the study of protein binding interaction of daspone and pyrimethamine [9]. Structure based approach for discovering protein–ligand binding affinity and drug designing from serum albumin model systems studied using NMR technique [10]. Affinity and specificity of ciprofloxacin-BSA interaction determined by fluorescence spectrophotometry [11]. Mass spectrometry based tools used to investigate protein-ligand interactions for drug discovery [12]. Interaction of propanolol with glycoprotein deliberated using micro liquid-liquid interface [13]. Comparative study of various techniques for drug-protein binding gives informative knowledge [14]. Study of interaction of the bioactive component Jatrorrhizine to HSA shows significant change in secondary structure of HSA [15]. Interactions of HSA with chlorogenic acid and ferulic acid observed [16]. Study of binding of atrazine and 2, 4-D with HSA show partial unfolding [17]. Effect of binding of mitoxantrone with HSA was successfully observed using FT-IR spectroscopy [18]. Quercetin and amantadine successfully binds with egg albumin which form new complex [19].

In this paper we report the simple and useful ultrasonic interferometer technique for the study of interaction of carboxamide derivatives of amino acid with BSA. The compounds synthesized are biologically active and shows pharmaceutically importance due to its antibacterial activity [20]. The binding affinity of these compounds with BSA measured using ultrasonic interferometer and effect of pH on binding affinity also measured by acoustical properties.

2. Results and Discussion

The carboxamide derivatives of amino acids were synthesized using known method [20] and characterized by spectral techniques viz. IR, NMR and Mass spectrometry (**Fig. 1**).

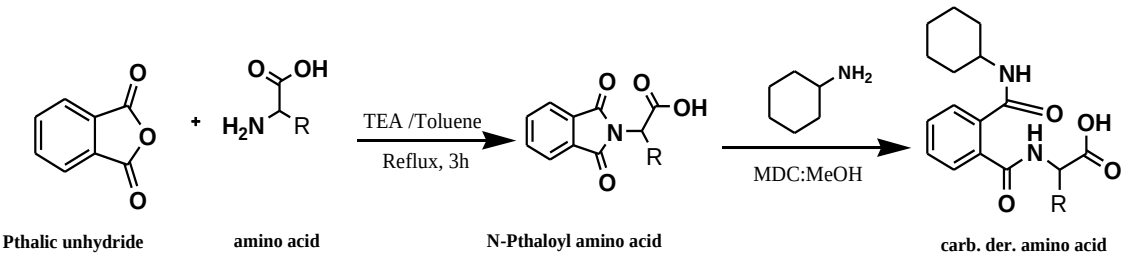

Figure 1. Scheme for synthesis of carboxamide derivatives of amino acids. Where, R= H, -CH_3, -CH-$(CH_3)_2$, -CH2-CH-$(CH_3)_2$, -CH_2-CH_2-S-CH_3. 2-Benzamido-4-methylpentanoic acid-2-cyclohexyl carboxamide (**2-BMCA**), 2-{[2-(cyclohexycarbamoyl) benzoyl] amino}-4-(methylsulfanyl) butanoic acid (**2CA4MBA**), 2-{[2-(cyclohexylcarbamoyl) benzoyl] amino}-3-methylbutanoic acid(**2CA3MBA**), 2-{[2-(cyclohexycarbamoyl) benzoyl] amino} propanoic acid(**2CMPA**), and 2-Benzamido acetic acid 2-cyclohexyl carboxamide (**2BA2C**).

Experimental observations

50 uM solution of BSA and 0.01M solution of compounds are prepared using acetate buffer solution of pH 3, 4 and 5. Complex solution of BSA and compounds were taken in a cell of 1MHz frequency of ultrasonic interferometer at various compositions viz. 9:1, 8:2, 7:3, 6:4, 5:5, 4:6 and ultrasonic velocity recorded for them. The values of ultrasonic velocities complex solutions of compounds 2CA3MBA, 2CA4MBA, 2BMCA, 2BA2C, 2CMPA with BSA are recorded at pH 3, 4 and 5 which are shown in **Table 1, 2** and **3**, respectively.

Measurement of ultrasonic velocity at varying composition of BSA and compounds at pH 3, 4 and 5 gives the value of association constant (K_f) which is calculated from Scatchard graph. The value of association constant (K_f) for complex solutions of the compounds at pH 3, 4 and 5 are: **2CA3MBA** 0.5035, 0.5023 and 0.5021, for **2CA4MBA** 0.5052, 0.5038 and 0.5032, for **2BMCA** 0.5036, 0.5024 and 0.5023 for **2BA2C** 0.5042, 0.5024 and 0.5022 and for **2CMPA** 0.5041, 0.5023 and 0.5017, respectively. **Fig. 2** shows graph of ultrasonic velocity vs Percent ligand fraction and specific binding vs percent ligand fraction for all the compounds at pH 3. **Fig.**

3 and **Fig. 4** shows graph of ultrasonic velocity vs Percent ligand fraction and graph of Specific binding vs Percent ligand fraction for all the compounds at pH 4 and 5.

Table 1. Ultrasonic velocities for compounds at pH 3.

Composition of BSA-Compounds	Ultrasonic velocity at pH 3				
	2CA3MBA	2CA4MBA	2BMCA	2BA2C	2CMPA
10:0	1484.700	1484.687	1485.870	1485.270	1485.270
9:1	1512.260	1510.860	1506.260	1508.529	1507.870
8:2	1519.870	1511.370	1509.870	1509.189	1508.529
7:3	1520.260	1516.260	1510.260	1509.580	1509.580
6:4	1518.920	1518.920	1508.920	1510.512	1509.850
5:5	1519.870	1519,670	1510.870	1509.870	1510.570
4:6	1520.870	1519.870	1510.870	1510.512	1508.529

Table 2. Ultrasonic velocities for compounds at pH 4.

Composition of BSA-Compounds	Ultrasonic velocity at pH 4				
	2CA3MBA	2CA4MBA	2BMCA	2BA2C	2CMPA
10:0	1492.860	1494.260	1494.260	1495.877	1495.877
9:1	1514.870	1509.270	1504.870	1508.280	1508.529
8:2	1518.928	1513.728	1508.928	1509.870	1509.129
7:3	1521.260	1513.260	1511.260	1511.211	1509.850
6:4	1522.860	1514.727	1511.860	1512.529	1510.512
5:5	1519.870	1515.540	1509.870	1510.870	1510.850
4:6	1518.684	1515.884	1510.684	1511.684	1510.512

Table 3. Ultrasonic velocities for compounds at pH 5.

Composition of BSA-Compounds	Ultrasonic velocity at pH 5				
	2CA3MBA	2CA4MBA	2BMCA	2BA2C	2CMPA
10:0	1495.207	1495.707	1498.327	1497.247	1497.280
9:1	1519.260	1511.260	1509.260	1509.260	1506.529
8:2	1522.870	1512.189	1513.870	1513.163	1507.824
7:3	1522.698	1512.698	1512.698	1513.827	1508.590
6:4	1524.260	1514.512	1514.260	1513.499	1509.120
5:5	1525.870	1516.260	1514.860	1513.836	1509.590
4:6	1525.260	1515.860	1513.870	1513.512	1509.870

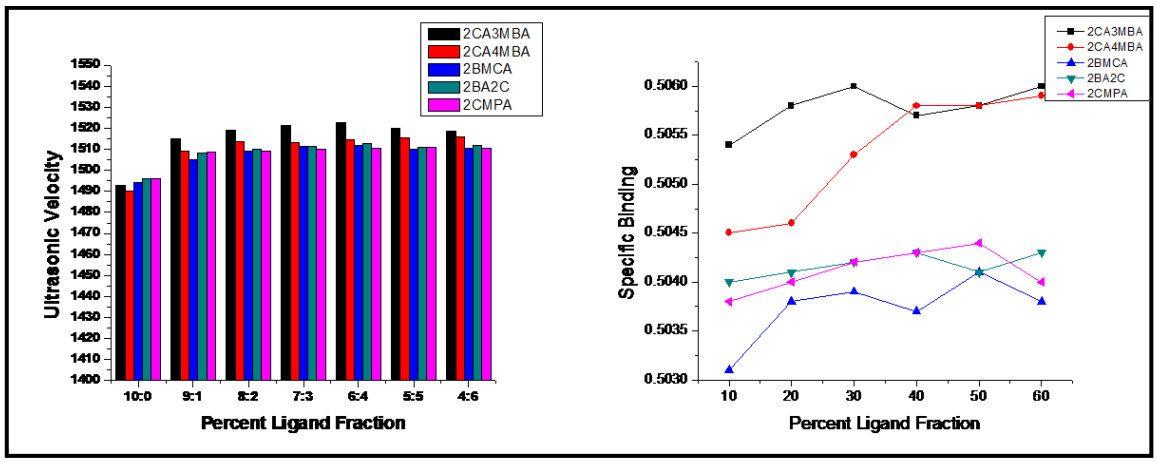

Figure 2. Graph of Ultrasonic velocity and Specific binding Vs Percent ligand fraction at pH 3 for carboxamide derivatives of amino acid.

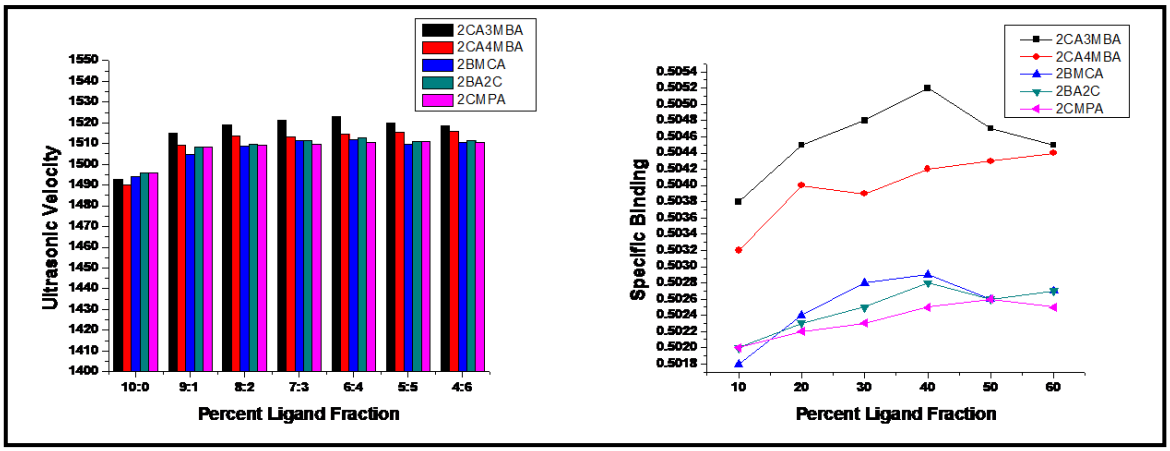

Figure 3. Graph of Ultrasonic velocity and Specific binding Vs Percent ligand fraction at pH 4 for carboxamide derivatives of amino acid.

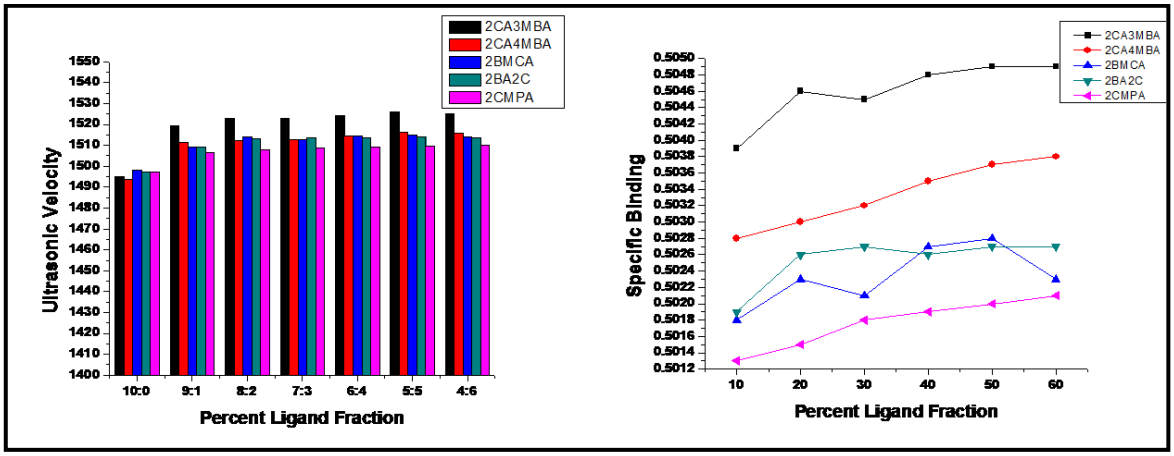

Figure 4. Graph of Ultrasonic velocity and Specific binding Vs Percent ligand fraction at pH 5 for carboxamide derivatives of amino acid.

3. Material and Methods

Synthesis

For the synthesis of the compounds all the chemicals used were of A.R. grade of Merck India Limited make and purchased from commercial suppliers. The purity of the synthesized compound was as certain by thin layer chromatography on silica gel G in petroleum ether and ethyl acetate (7:3) mixture, melting point was recorded using digital melting point apparatus Equiptronics (EQ 730). ^{1}H NMR spectra of the compound were recorded in $CDCl_3$ on NMR instrument (500MHz) using TMS as an internal standard from SAIF, CDRI Lucknow.

For measurement of binding, digital ultrasonic echo pulse velocity meter (Vi Microsystem Ltd. India), BSA (M_r = 66,500) (Chemsworth chemical Ltd. India), 0.1M sodium acetate buffer solution of 3, 4 and 5 (± 0.05) pH was used.

Measurement of binding affinity

Ultrasonic interferometer was set up at 1MHz frequency range and appropriate cell of the frequency used. BSA solution of 50µM concentration in aqueous phase using acetate buffer of pH range 3,4 and 5 prepared and ultrasonic velocity of these solutions were calculated in lack of compounds. Secondly the solution of compounds of 0.01M concentration prepared using buffer solution of varying pH 3, 4 and 5. Mixture of solution of BSA and compounds at pH 3 in different composition viz. 9:1, 8:2, 7:3, 6:4, 5:5, 4:6 prepared and used to measure ultrasonic velocities. Similarly, ultrasonic velocities for same composition of BSA and compound at 4 and 5 pH were also recorded. *Scatchards plot* used for measurement of specific binding of compounds with BSA at different composition and from which the value of association constant are calculated.

4. Conclusions

Carboxamide derivatives of amino acids shows antibacterial activity hence they considered as pharmaceutically active compounds. For drug showing pharmaceutical activity, it is a significant aspect to see their affinity towards plasma protein. The interaction of these amino acid derivatives observed with bovine serum albumin (BSA) under the condition of varying pH and temperature using the ultrasonic interferometer technique. After interaction between BSA-carboxamide derivatives of amino acid, different association constant values at different pH obtained. It is observed that the value of the association constant decreases with an increase in the pH value for all the compounds 2CMPA, 2BA2C, 2CA3MBA, 2-BMCA and 2CA4MBA at pH 3, 4, and 5 respectively. It means the order of the association constant for all the compounds at pH 3, 4, and 5 is 3 >4 > 5.

But the order of the association constant varies for compounds at varying pH values is slightly different. At pH 3 the sequential order of the association constant for compounds is 2CA4MBA > 2BA2C > 2CMPA > 2BMCA > 2CA3MBA.

At pH 4 the order of the association constant for compounds is 2CA4MBA > 2BA2C > 2BMCA > 2CMPA > 2CA3MBA.

At pH 5 the sequence order of the association constant for compounds is 2CA4MBA > 2BMCA > 2BA2C > 2CA3MBA > 2CMPA.

So, there is slight variation observed for compounds while considering the effect of each pH separately on each drug. At all the pH the value of the association constant is highest for compound 2CA4MBA which is may be due to long alkyl chain having sulphur atom sandwiched in it, sulphur increases the activity of compound

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