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BSA binding of 2-(aminomethyl)cyclopropane-1,1-dicarboxylic acid

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Abstract: Herein, we present the results of the study devoted to the exploration of BSA binding of 2-(aminomethyl)cyclopropane-1,1-dicarboxylic acid, as an example of constrained γ-amino dicarboxylic acid, and, taking into consideration that the effectiveness of a potential drug depends on its ability to bind to a protein carrier and in that way enable transfer through the blood stream. For the investigation of binding properties, we used the fluorescence emission titration of BSA with a synthesized compound. Considering that the BSA solution shows an intensive fluorescence emission around 360 nm, a decrease in emission intensity at λ = 366 nm with the addition of a solution of 2-(aminomethyl)cyclopropane-1,1-dicarboxylic acid indicated the binding of the tested compound. According to the results obtained, our compound binds to the BSA in a molar ratio 1:1 ($n \approx 1$). The optimal values of binding constant Ka are between 10^4 and 10^6 M^{-1}, which indicates to us that the Ka value of the tested compound is in the favorable range.

Keywords: amino acids, cyclopropanes, BSA

1. Introduction

The amino acids have gained important relevance in the research world particularly their unnatural counterparts as constituents of molecules with promising pharmaceutical potential [1]. The replacement of natural amino acids in peptides with non-proteinogenic examples has inspired the development of different studies directed to the better understanding of interactions of small molecules with biological targets such as enzymes or receptors [2, 3]. One of the most important fields in drug development is the design and synthesis of peptidomimetic molecules that should have the same pharmaceutical properties as their natural counterparts, but much better metabolic stability. For this purpose, one of the widely accepted methodologies is the use of conformationally constrained amino acids and dipeptides as entities that mimic parts of natural peptidic substrates and that enable us to understand the relationships between

peptide conformation and biological activity. All these circumstances have caused the development of the synthetic methodologies devoted to the preparation of different examples of conformationally constrained amino acids and their analogs [4]. The examples of rigid cyclic amino acids have played an important role in therapeutic development due to their ability to, upon incorporation into peptides or peptidomimetics, induce conformational restrictions and enable significant structural effects [4]. Herein, we present the results of the *in vitro* study devoted to the exploration of BSA binding of 2-(aminomethyl)cyclopropane-1,1-dicarboxylic acid 1 (Figure 1), as an example of sterically constrained amino acid derivatives and taking into consideration that the effectiveness of a potential drug depends on its ability to bind to a protein carrier and in that way enable transfer through the bloodstream.

2. Experimental section

2.1. Synthesis of 2-(aminomethyl)cyclopropane-1,1-dicarboxylic acid

The synthesis of racemic 2-(aminomethyl)cyclopropane-1,1-dicarboxylic acid 1 was achieved using the previously described procedure in the literature [5].

2.2. Albumin-binding studies

The protein-binding studies were made in accordance with the method described in the literature [6-8].

3. Results and Discussions

Since the effectiveness of a potential drug depends on its ability to bind to a protein carrier, which plays a key role in the transfer of substances through the blood stream, the binding affinity of compound 1 to bovine serum albumin (BSA) has been examined.

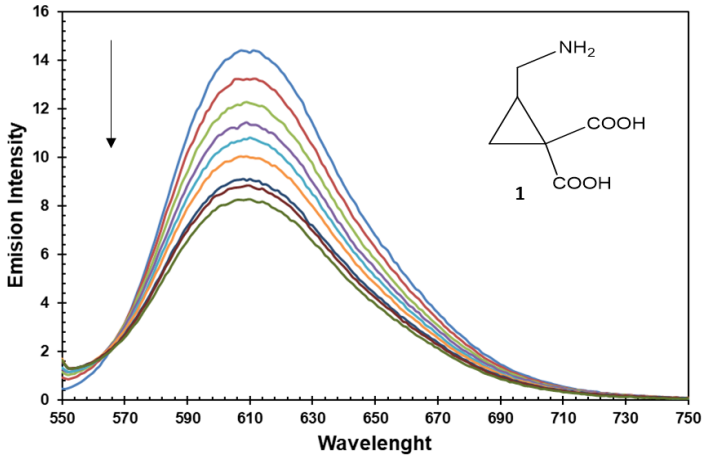

Figure 1. Emission spectra of BSA in the presence of 2-(aminomethyl)cyclopropane-1,1-dicarboxylic acid. [BSA] = 2 μM, [Acid]/ [BSA] = 0-10, λ_{ex} = 295 nm. Arrows show the intensity changes upon increasing the concentrations of the examined compound.

For the investigation of binding properties, we used the fluorescence emission titration of BSA with synthesized compounds. The spectra were observed in the range between 300 and 500 nm at an excitation wavelength of 295 nm. Considering that the BSA solution shows an

intensive fluorescence emission around 360 nm, at the mentioned wavelength [9], decrease in emission intensity at λ = 366 nm (Figure 1) with the addition of a solution of 2-(aminomethyl)cyclopropane-1,1-dicarboxylic acid indicated binding of tested compound to the BSA molecule.

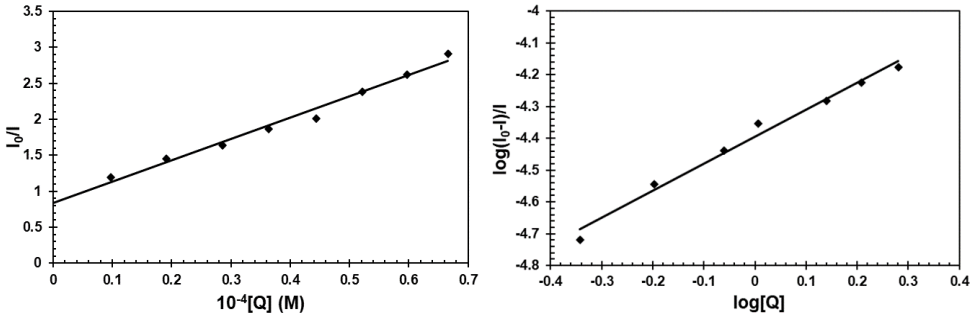

Figure 2. left: Plots of I_0/I versus [Q] in mol/dm^3; right: lots of log(I_0-I/I) versus log [Q].

The fluorescence quenching data such as Stern-Volmer constant (K_{sv}) were defined from the linear dependence of I_0/I to the concentration of the tested compound (Figure 2, left) using the Stern-Volmer quenching equation and number of binding sites per BSA molecule (n) were acquired by using the Stern-Volmer equation (1)

$$I_0/I = 1 + K_{sv}[Q] \qquad (1)$$

where I_0 and I are the emission intensities in the absence and in the presence of the quencher (tested compound), [Q] is the quencher's concentration.
Binding constant (Ka) can be calculated from the equation 2, which represents the relationship between fluorescence quenching intensity and the concentration of quencher [8]

$$\log[(I_0 - I)/I] = \log Ka + n \cdot \log[Q] \qquad (2)$$

where n is the number of binding sites per BSA. The value for Ka can be calculated through the linear dependence of log [(I_0 - I)/I] versus log [Q] (Figure 2, right), where Ka can be calculated from the slope, while the n represents the intercept.

The calculations of all parameters were performed, and the obtained values are presented in Table 1.

Table 1. Binding parameters (K_{sv}, Ka, n) and the correlation coefficient (R) for interactions of the tested compound with BSA.

	$10^4 K_{sv}$ BSA (M^{-1})	Ka [M^{-1}]	R	n
Acid 1	5.7 ± 0.1	1.8 x 10^5	0.99	1.02

If we look at the Ka value in Table 1, we can conclude that the tested compound has a very good binding affinity to BSA molecules, knowing that the optimal values of binding constant Ka are between 10^4 and 10^6 M^{-1} [9]. Also, we can conclude that our compound binds to the BSA in molar ratio 1:1 (n ≈ 1). The optimal values of binding constant Ka are between 10^4 and 10^6 M−1 [9], which indicates to us that the Ka value of the tested compound is in the favorable range.

4. Conclusions

According to the results obtained in the screening of binding affinity of 2-(aminomethyl)cyclopropane1,1-dicarboxylic acid toward BSA, we can conclude that our compound has a very good binding affinity toward BSA molecules and that it binds to the BSA in molar ratio 1:1.

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