Orbital: The Electronic Journal of Chemistry
journal homepage: www.orbital.ufms.br
e-ISSN 1984-6428

| Vol 11 | | No. 3 | | April-June 2019 |

FULL PAPER

Thermodynamic Study of Comfarol Binding to Urease

Leila Zolghadr[a], Gholamreza Rezaei Behbehani[a]*, Adeleh Divsalar[b], and Zahra Moubarakabadi[a]

[a]Department of Chemistry, Imam Khomeini International University, Qazvin, Iran.
[b]Department of Cell and Molecular Sciences, Kharazmi university, Tehran, Iran.

Article history: Received: 09 December 2018; revised: 03 June 2019; accepted: 20 June 2019. Available online: 29 June 2019. DOI: http://dx.doi.org/10.17807/orbital.v11i3.1369

Abstract:

Isothermal Titration Calorimetry, ITC, was used to study of urease, binding with comfarol in phosphate buffer at pH 7.4. Data analyzing of comfarol interaction with urease was performed by the extended solvation model and the positive cooperativity of comfarol with urease indicates that comfarol causes stabilization of the urease structure.

Keywords: comfarol; the extended solvation model; urease

1. Introduction

Urease inhibitors play the role of potential drugs in serious infections caused by Proteus and related species in the urinary tract, as well as Helicobacter pylori in the gastrointestinal tract.

Urease, the enzyme responsible for the rapid hydrolysis of urea to ammonia, is highly stable in aqueous solutions and resistant to nonenzymatic breakdown. In gastric tract infection, urease provides ammonia for bacterial protein synthesis and helps in the colonization of the host by neutralizing gastric acid. Continuing to neutralize acid locally and shedding urease cause defect in host defense mechanisms [1- 3]. There are reports have suggested that urease-producing bacteria play the main role in the formation of infection-induced urinary stones and by supersaturation with respect to struvite and calcium phosphate [4- 8].

In this research work we have applied the most predictive theory of extended solvation model to study the thermodynamic parameters of comfarol+urease interaction. The results obtained from this investigation represents positive cooperativity of comfarol with urease. From the thermodynamic parameters, ΔH and ΔS, it was concluded that hydrophobic forces are dominant.

2. Results and Discussion

Previously, we have demonstrated that the heats of the biomolecules+ligands interactions (q), in the aqueous solvent system can be calculated successfully using the following equation [9 - 11]:

$$q = q_{max}x'_B - \delta_A(x'_A L_A + x'_B L_B) - (\delta_B - \delta_A)(x'_A L_A + x'_B L_B)x'_B \quad (1)$$

where q are the heats of comfarol + urease interactions, and q_{max} represents the heat value upon saturation of all JBU. The JBU stability in the low and high comfarol concentrations are shown by δ_A and δ_B parameters, respectively. The positive values for δ_A and δ_B represent the JBU stabilization by the ligands, while negative values of δ_A and δ_B indicate that comfarol stabilized the JBU structure.

Cooperativity results from the interactions between identical binding sites with the same ligand, so cooperative binding requires macromolecule with more than one binding site.

*Corresponding author. E-mail: grb402003@yahoo.com

If binding of a ligand molecule increases the receptor's apparent affinity, cooperativity can be positive, and hence increases the affinity of another ligand molecule binding, which is shown by p>1. *p*<1 indicate that the binding of the ligand molecules have less affinity for binding to the other sites on the biomolecule, can be appoint negative cooperativity. *p*=1 demonstrates the noncooperative interaction.

Equation 2 express x'_B as follows:

$$x'_B = \frac{p x_B}{x_A + p x_B} \qquad (2)$$

where x'_B , and $x'_A = 1 - x'_B$ are the fraction of bound and unbound ligand, respectively. x_B fractions was calculated by equation 3 from ligand concentrations, after each injection divided by the maximum concentration of the ligand upon saturation of all JBU, $[ligand]_{max}$ as follows:

$$x_B = \frac{[ligand]}{[ligand]_{max}} \qquad (3)$$

L_A and L_B are the relative contributions of unbound and bound Ligand in the dilution heats of ligand in the absence of JUB. Fitting of the heats of JUB+ comfarol interactions was performed across the entire comfarol. In the fitting procedure, p was changed until the best agreement between the experimental and calculated data was approached (Figures 1).

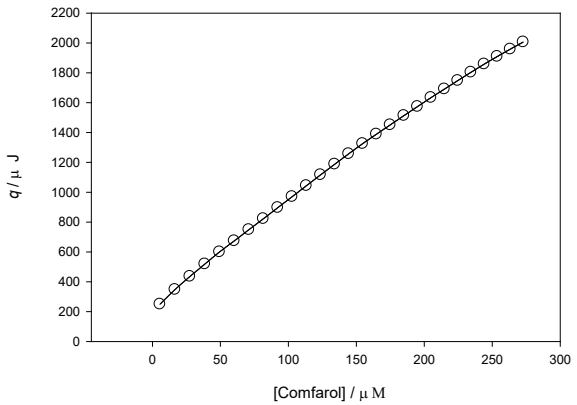

Figure 1. Comparison between the experimental heats (▵) at 300 K, for (JUB+ comfarol)
The high r^2 value (0.999) supports the method.

The binding parameters for JUB+comfarol, obtained from equation 1, were reported in Table 1. The agreement between the experimental and theoretical calculation results significantly proves the equation 1.

Table 1. Binding parameters for JBU+Comfarol interaction.

parameters	p	g	Ka/L mol^{-1}	ΔH/kJ mol^{-1}	ΔG/kJ mol^{-1}	TΔS/kJ mol^{-1}	δ_A	δ_B
	1.2	12	253009	5.16	-31.03	36.19	-0.23	-0.17

As indicated by the binding parameters, the interaction is entropy-driven indicating that the hydrophobic forces are dominant. δ_A and δ_B values are very closed together, indicating so little changes in JBU structure as result of its interaction with comfarol. Negative δ_A and δ_B values are indicative of formation of unstable complex of JBU with Comfarol.

Small changes in δ_A and δ_B values is the characteristic of specific interaction and it is possible to conclude that the most of JBU is in its native state. *p*>1(p=1.2 in Table 1) as well as negative δ_A and δ_B, indicates that comfarol causes a little reversible changes in the JBU structure. The weak interaction of comfarol with JBU, suggest that this drug is not suitable for urease inhibition.

Consider a biomolecule, with η binding sites for ligands. The binding of the ligands to the biomolecule can be represented by the chemical equilibrium expression:

$$P + nL \Leftrightarrow PL_n \qquad (E1)$$

K_A is the ligand concentration, in which the ligand occupying half of the binding sites. Because K_D is defined so that $K_D = (K_A)^\eta$, this is also known as the microscopic dissociation constant, where $K_D = \frac{[P][L]^n}{[PL_n]}$. Substitution of $\frac{(1-\theta)}{\theta} = \frac{[P]}{[PL_n]}$ in $K_D = \frac{[P][L]^n}{[PL_n]}$, we will approach to:

$$\frac{\theta}{(1-\theta)} = \frac{[L]^n}{K_D}$$

Taking the logarithm of both sides of the equation leads to an alternative formulation of the Hill equation:

$$Log\left(\frac{\theta}{1-\theta}\right) = n\,Log[L] - \log(K_D) \qquad (5)$$

Assuming that $\theta = \frac{q}{q_{max}}$ is the fraction of the ligand binding sites on the biomolecule which are occupied by the ligand, we will arrive to a similar equation of Hill [12] as follows:

$$Log\left(\frac{q_{max}-q}{q}\right) = n\,LogK_a - n\,Log\,[\text{comfarol}] \qquad (6)$$

The number of comfarol around JBU, *n*, and association equilibrium constant, K_A, were determined graphically on the basis of equation 6. The Gibbs free energies can be obtained as follows:

$$\Delta G = -RT\ln K_a \qquad (7)$$

The values of *n* in both two comfarol concentration regions (Table 2) are more than one, which suggests that the binding of one molecule of comfarol to JBU increases affinity of comfarol for binding to other binding sites.

Equation 1 is able to predict the heat of the interaction over the whole range of comfarol concentrations, while equation 6 can not predict the data in the whole range of comfarol concentration in one step. Therefore, in order to use equation 6, it is necessary to separate the data in two series, one set in the low concentrations and the second set in the high concentrations of comfarol. Clearly, the precision of binding parameters calculated from equation 1 are much more than the results arrived from equation 6.

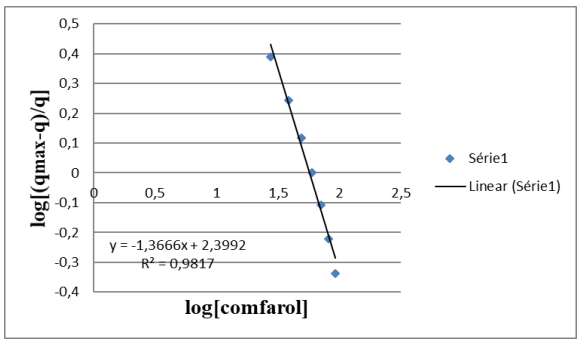

Figure 2. The fitting of heats of comfarol+JBU interactions in the low concentration of comfarol.

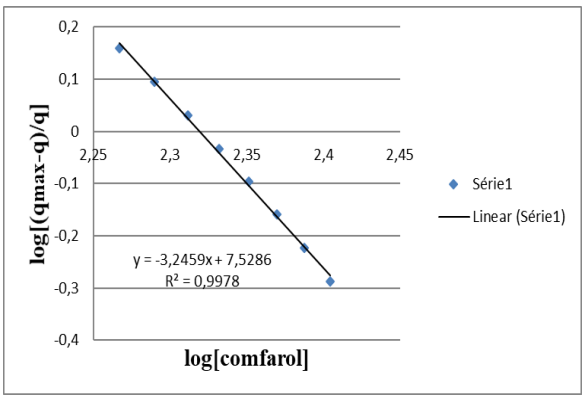

Figure 3. The fitting of heats of comfarol+JBU interactions in the high concentration region of comfarol.

Table 2. Thermodynamic parameters for comfarol+JBU interactions approached from Eq. 2.

Thermodynamics parameters	*n*	K_a/M^{-1}	ΔH/kJmol^{-1}	ΔG/kJmol^{-1}	$T\Delta S$/kJmol^{-1}
First series	1.37	5.65 x	4.52	-44.62	49.15
Second series	3.25	2.46 x	4.52	-48.31	52.83

There are 2 set of biding series. *n*>1 in both two regions indicate positively cooperative binding, which is in agreement with the results obtained from equation 1. The interaction is entropy-driven, indicating that the hydrophobic forces increase the stability of JBU.

3. Material and Methods

Comfarol, 90% (HPLC), were purchased from Sigma chemical Co. and Jack bean urease was obtained from Sorachim. Solutions were made in 50 mmol L^{-1} buffer phosphate using double-distilled water, and all other materials and reagents were of analytical grade.

A four-channel commercial microcalorimetric system, Thermal Activity Monitor 2277, Thermometric, Sweden, was used to perform

isothermal titration microcalorimetric experiments. All solutions were degassed, by stirring under vacuum, before being used. The comfarol solution (8000 μmol · L^{-1}) was injected into the calorimetric titration vessel, which contained 1.8 mL JBU (5 μmol · L^{-1}) by use of a Hamilton syringe.

Permanently, a thin stainless steel hypodermic needles with 0.15 mm inner diameter which was fixed to the syringe, reached straight into the calorimetric vessel. The titration of JBU with camfarol solution involved 30 consecutive injections of the ligand solution and the first injection was 5μL and the remaining ones were 10 μL. The microcalorimeter was frequently calibrated electrically during the experiment. The digital voltmeter which was applied to measure the calorimetric signal was part of a computerized recording system. By the "Thermometric Digitam 3" software program, the heat of each injection was calculated. The heat of dilution of the comfarol solution was measured as well, except that JBU was excluded. In other words, heats of dilution of the Kaempferol solutions were subtracted from the heat of comfarol+JBU interaction and the heats of dilution of JBU are negligible.

4. Conclusions

Comfarol has high affinity for binding to urease and decreases urease activity. It is possible to conclude that the inhibition of urease by confarol is competitive and there is a little change in urease structure.

References and Notes

[1] Macegoniuk, K.; Kowalczyk, R.; Rudzin´ ska, A.; Psurski, M.; Wietrzyk, J.; Berlicki, L. *Bioorg. Med. Chem. Lett.* **2017**, *27*, 1346. [Crossref]

[2] Ntatsopoulos, V.; Vassiliou, S.; Macegoniuk, K.; Berlicki, L.; Mucha, A. *Eur. J. Med. Chem.* **2017**, *133*, 107. [Crossref]

[3] Griffith, D. P.; Musher, D.M.; Itin, C. *Invest. Urol.* **1976**, *13*, 346.

[4] Benini, S.; Kosikowska, P.; Cianci, M.; Mazzei, L.; Vara, A. G.; Berlicki, L.; Ciurli, S. *J. Biol. Inorg. Chem.* **2013**, *18*, 391. [Crossref]

[5] Kosikowska, P.; Berlicki, L. *Expert Opin. Ther. Patents* **2011**, *21*, 6. [Crossref]

[6] Berlicki, L.; Bochno, M.; Grabowiecka, A.; Białas, A.; Kosikowska, P.; Kafarski, P. *Amino Acids* **2012**, *42*, 1937. [Crossref]

[7] Yean-Jang, L.; Tsao-Dong, W. *J. Chin. Chem. Soc.* **2001**, *48*, 201.

[8] Mansoor, F.; Asnis, I. C. *J. Chem. Soc. Pak.* **2013**, *35*, 233.

[9] Rezaei Behbehani, G.; Saboury, A. A.; Taherkhani, A.; Barzegar, L.; Mollaaghazade, A. *J. Therm. Anal. Calorim.* **2011**, *105*, 1081. [Crossref]

[10] Rezaei Behbehani, G.; Saboury, A. A.; Taleshi, E. *J. Mol. Recognit.* **2008**, *21*, 132. [Crossref]

[11] Rezaei Behbehani, G.; Saboury, A. A.; Barzegar, L.; Zarean, O. *J. Therm. Anal. Cal.* **2010**, *101*, 379. [Crossref]

[12] Ortiz, A. *Journal of Uncertain Systems* **2013**, *7*, 198. [Link]