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

| Vol 11 | | No. 7 | | October-December 2019 |

FULL PAPER

A Thermodynamic Study on the Binding of Polyethyleneglycol 1500 Stearic Acid with Lysozyme

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Article history: Received: 18 December 2018; revised: 02 November 2019; accepted: 12 November 2019. Available online: 30 December 2019. DOI: http://dx.doi.org/10.17807/orbital.v11i7.1373

Abstract:

Thermodynamics of the interaction between copolymer of Stearic acid + polyethyleneglycol 1500 mixtures, S1500, with lysozyme was investigated at pH 7.0 and 27 °C in phosphate buffer by isothermal titration calorimetry, ITC. The extended solvation model was used to reproduce the enthalpies of S1500+lysozyme interactions. The solvation parameters recovered from the extended solvation model, attributed to the structural change of lysozyme. The binding parameters found for the interaction of S1500 with lysozyme, indicate that there are 2 set of binding sites in this interaction. The observations indicated that the low S1500 content induced protein stabilization, whereas at the high S1500 concentration, much more stabilization occurred in lysozyme structure.

Keywords: lysozyme; polyethyleneglycol 1500; stearic acid

1. Introduction

Six, cavity-creating mutants were constructed within the hydrophobic core of lysozyme. The substitution of these cavities decreased the stability of the lysozyme. The hydrophobic effect is usually considered as the major factor in determining the stability of the folded structures of globular proteins. Mutations with the creation of larger cavities may induce substantial changes in the structure of a protein. In such cases, hydrophobic effect cannot be predicted by just considering specific residues involved in the mutation. In other words, the same type of substitution has been found to give a wide range of changes in the free energy of folding for different mutant structures. The energy term that increases with the size of the cavity can be expressed either in terms of the cavity volume or in terms of the cavity surface area. The previous reports indicated the possibility of extending the range of lysozyme activity to include Gram-negative bacteria, using thermal modification. It has also been found that heat denaturation of lysozyme caused by increasing temperatures results in the progressive loss of enzymatic activity, while its antimicrobial action towards Gram negative bacteria is greatly enhanced. In the last few years, aqueous polymer solutions of the polyethyleneglycol, PEG, family have been widely applied mostly because of their use in removing macromolecules from a natural complex mixture [1]. It has been found that PEG has bio protectant effects in interacting with protein molecules [2]. Precise and versatile application of PEG in proteomics and other biological research methods depends upon the availability of polyethylene glycol derivatives of defined length (MW) that are activated with specific functional groups. Some reports showed that poly (l-histidine) component of PEG-polyhistidine may be physically attached to the anionic surfaces of different proteins such as BSA (bovine serum albumin) and insulin through ionic interactions

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with minimal disturbance of protein's tertiary structure [3]. Hydrophilic polyethyleneglycol is proposed to cover the surface of the protein acting as the corona. This feature may have prevented protein interfacial adsorption and reduced the extent of denaturation of proteins [4-10].

Various proteins in the cell membrane are in complex interaction with lipids containing different types of fatty acids. The complexes formed between protein-polyelectrolytes play an important role in many chemical and biological processes, such as protein separation, enzyme stabilization and drug delivery. So far, many studies have been carried out to investigate the characteristics of diblock copolymers but there are few reports on the effect of diblock copolymers on the structure and stability of globular proteins, such as lysozyme.

Lysozyme with a molecular weight of 14400 kDa (129 amino acid residues) is a basic protein belongs to the class of enzymes that lyse the cell walls of bacteria [7-11]. Reports showed that antimicrobial effects of lysozyme against gram-negative bacteria are increased by covalent binding to palmitic acid. Spectroscopic studies of hen egg lysozyme-glucose stearic acid monoester conjugate, revealed that the α-helix content was slightly lower but the conformation around Trp had not changed. It is interesting to point out that the conjugate maintained around 53-57% of the enzymatic activity of native hen egg lysozyme at 40-60 °C and exhibited considerable resistance to proteolysis [9-11].

In this research, thermodynamic investigations of the interaction between Stearic acid + polyethyleneglycol 1500, S1500 and lysozyme were performed. It is important to mention that this is the first attempt to estimate the thermodynamic parameters due to such interactions by isothermal titration calorimetric experiments. The obtained results indicate that S1500, which had a high hydrophobicity, coexisting with the lysozyme could show a higher antimicrobial activity than the pure lysozyme.

All of the Information obtained from this study will be helpful to provide useful information to design better drug carrier system in the future.

2. Results and Discussion

It has been shown previously [17-20] that the heats of the biopolymers + solvent (S1500+lysozyme in this case) interactions in the aqueous solvent mixtures, can be reproduced by:

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

In Eq. 1 q is the heats of S1500+lysozyme binding, and q_{max} the heat value upon saturation of all lysozyme. The parameters δ_A and δ_B reflect the net effects of S1500 on the lysozyme stability in the low and high S1500 concentrations respectively, with positive δ_A and δ_B values indicate that S1500 stabilizes lysozyme structures. x'_B can be expressed as follows:

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

p<1 or p>1 indicate positive or negative cooperativity of macromolecule for binding with ligand respectively; p = 1 indicates that the binding is non-cooperative. If the ligand binds at each site independently, the binding is non-cooperative. x_B is the fraction of bounded S1500 to the binding sites, $x_A = 1 - x_B$ is the fraction of unbounded S1500. We can express x_B fractions, as the total S1500 concentrations divided by the maximum concentration of the S1500 upon saturation of all lysozyme as follows:

$$x_B = \frac{[S1500]}{[S1500]_{max}} \quad (3)$$

$[S1500]$ is the concentration of S1500+PEG after every injection and $[S1500]_{max}$ is the maximum concentration of the S1500 upon saturation of all lysozyme. In general, there will be n sites for binding of S1500 per lysozyme molecule. L_A and L_B are the relative contributions of unbounded and bounded S1500 in the heats of dilution with the exclusion of lysozyme and can be calculated from the heats of dilution of S1500-PEG in the buffer solution, q_{dilut}, as follows:

$$L_A = q_{dilut} + x_B\left(\frac{\partial q_{dilut}}{x_B}\right), \quad L_B = q_{dilut} - x_A\left(\frac{\partial q_{dilut}}{x_B}\right) \quad (4)$$

The heats of S1500+lysozyme interactions, q, were fitted to Eq. 1 across the whole range of S1500 concentrations. In the procedure, the only adjustable parameter (p) was changed until the best agreement between the experimental and

calculated data was approached (Fig. 1).

The binding parameters recovered from these analyses are listed in Table 1 and these values calculated via Eq. 1. Positive cooperativity in Table 1, indicates that polyethyleneglycol 1500+stearic acid stabilizes the lysozyme structure.

Table 1. Thermodynamic parameters for S1500+lysozyme interactions in 1mM S1500 solution approach from Eq. 1.

[lysozyme]	*p*	δ_A^o	δ_B^o
75 μ M	1.20	0.41	0.66

Using these parameters are compared with the experimental data in Fig.1. The agreement between the calculated and experimental results is excellent and gives considerable support to the use of Eq. 1. Consider a biomolecule, with n binding sites for ligands. The binding of the ligands to the biomolecule can be represented by the chemical equilibrium expression:

$$P + nL \Leftrightarrow PL_n$$

Assuming that $\frac{q}{q_{max}}$ is the fraction of the ligand binding sites on the biomolecule which are occupied by the ligand, it is reasonable to write the following Equation 5:

$$Log(\frac{q_{\max} - q}{q}) = n\ LogK_a - n\ Log\ [\mathrm{S1500}] \quad (5)$$

where K_a (forward rate, or the rate of association of the protein-ligand complex).

The number of S1500 around lysozyme n and association constant K_a were determined graphically on the basis of Equation 5.

K_a is the association equilibrium constant for S1500+lysozyme binding and n values obtained from the equation 5 were reported in Table 2.

The Gibbs free energies as a function of S1500 concentration can be obtained as follows:

$$\Delta G = -RT\ ln\ K_a \quad (6)$$

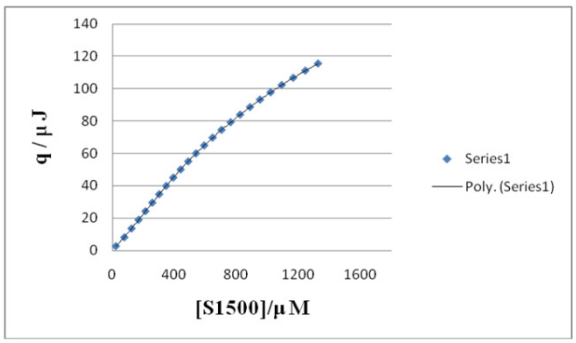

Figure 1. Comparison between the experimental heats, *q*, (ϒ), for S1500+lysozyme interactions and calculated data (lines) via Eq. 1.

Table 2. Thermodynamic parameters for S1500+lysozyme interactions in 1mM S1500 solution approach from Eq. 2.

Thermodynamics parameters	*n*	K_a / M^{-1}	ΔH / kJmol^{-1}	ΔG / kJmol^{-1}	$T\Delta S$ / kJmol^{-1}
First series	1.08	1.59×10^8	4.52	-47.22	51.75
Second series	3.12	1.38×10^8	4.52	-46.86	52.38

The low K_a values at the low S1500 concentrations reflect to the low affinity of lysozyme for S1500 in this domain (Table 2), that is in agreement with the less negative Gibbs free energies values in the low S1500 concentrations reflect to the low affinity of lysozyme for S1500 in this domain (Table 2), that is in agreement with the less negative Gibbs free energies values in the low S1500 concentrations in first and second series (Fig. 2 and 3) in this region.

It is possible to introduce a correlation between change in δ_A^θ and increase in the stability of lysozyme. The δ_A^θ value reflects the hydrophobic property of lysozyme, leading to the enhancement of water structure. The greater the extent of this enhancement, the greater the stabilization of the lysozyme structure and the greater the value of δ_A^θ. δ_A^θ and δ_A^θ values (Table 1) for lysozyme+S1500 interaction is positive, indicating that S1500 is the low and high concentrations regions has stabilized the lysozyme structure.

p Value is more than one (p =1.2), which indicates that there is the interaction is positive cooperativity.

The results obtaining from the interaction of

stearic acid+polyethyleneglycol and lysozyme probably provide useful information to design better drug carrier system in the future.

According to Table 2, there are 2 set of biding series. *n*>1 in both two regions indicate positively cooperative binding, which in agreement with results obtained from Equation 1. The interaction is entropy-driven, indicating that the hydrophobic forces increase the stability of lysozyme. The values of n in both two ligand concentration regions (Table 2) are more than one, which suggests that the binding of one molecule of stearic acid coexist with polyethyleneglycol 1500 to lysozyme increases affinity of stearic acid for binding to other binding sites.

3. Material and Methods

Hen egg-white lysozyme was obtained from Sigma and diblock copolymer of S1500 was synthesized. Protein concentrations were determined from absorbance measurements at 277 nm in 1 cm quartz cuvettes. The molar extinction coefficient of lysozyme was 7690 M^{-1} cm^{-1}. All other materials and reagents were of analytical grade, and solutions were made in 50 mM buffer phosphate using double-distilled water. The isothermal titration calorimetric experiments were carried out on a VP-ITC ultra-sensitive titration calorimeter (Micro Cal, LLC, Northampton, MA). The micro calorimeter consists of a reference cell and a sample cell of 1.8 mL in volume, with both cells insulated by an adiabatic shield. All solutions were thoroughly degassed before use by stirring under vacuum.

The sample cell was loaded with Lysozyme solution (75 μM) and the reference cell contained buffer solution. The solution in the cell was stirred at 307 rpm by the syringe (equipped with micro propeller) filled with diblock copolymer (S1500) solution (1mM) to ensure rapid mixing. Injections were started after baseline stability had been achieved. The titration of protein with diblock copolymer involved 30 consecutive injections of the ligand solution, the first being 5 μL, and the remaining ones of 10 μL. In all cases, each injection was done in 6 s at 3-min intervals. To correct the thermal effects due to diblock copolymer dilution, control experiments were done in which identical aliquots were injected into the buffer solution in the absence of lysozyme. In the ITC experiments, the enthalpy changes associated with processes occurring at a constant temperature are measured. The measurements were performed at a constant temperature of 27.0±0.02 °C and the temperature was controlled by using a Poly-Science water bath [12-17].

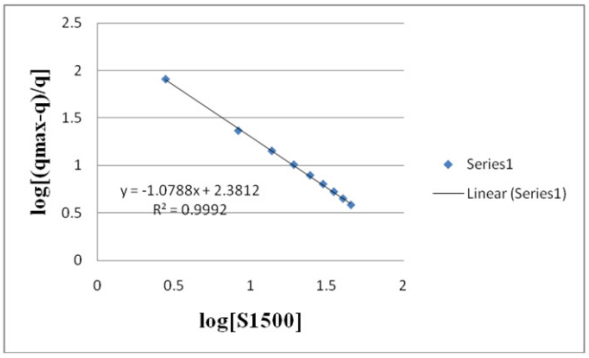

Figure 2. The fitting of heats of S1500+lysozyme interactions for the first set of binding series (in the low concentration of S1500).

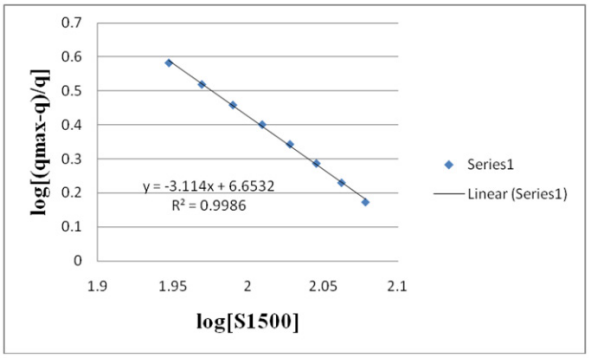

Figure 3. The fitting of heats of S1500+lysozyme interactions for the second set of binding series (in the high concentration of S1500).

4. Conclusions

The binding parameters found for the interaction of S1500 with lysozyme, indicate that there are 2 set of binding sites in this interaction. the observations indicated that the low S1500 content induced protein stabilization, whereas at the high S1500 concentration, much more stabilization occurred in lysozyme structure. It can be concluded that by increasing in molecular mass of PEG involved in a copolymer, the type and number of binding site of lysozyme for copolymer decreases might be represented by different structures (conformations) of protein in these interactions.

Acknowledgments

Financial support from the Universities of Imam Khomeini (Qazvin) is gratefully acknowledged.

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