**Biology and Biochemistry Department**

**BIOC 312**

**Biochemistry II lab**

**Experiment #1**

**Sec 1**

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**Title**

**Protein-ligand interaction – Binding of coomassie blue dye of ovalbumin**

* **Objective:**

 The purpose of this experiment is to learn about the properties Coomassie Brilliant Blue G-250dye of ovalbumin protein. These properties were measured using a spectrophotometer, and the higher the absorption, the lower the binding. Another objective is to generate a saturation curve and calculate the Amax, as well as a Scatchard plot that quantitatively describes the binding process.

* **Introduction:**

 An important reason for which many drugs have been developed or even made is molecular recognition, by which biological molecules interact with small molecules or even with each other. Proteins are large biological molecules and most living organisms speed up their processes through proteins, and one of the functions of proteins is that they bind to each other or to other molecules, which leads to a good understanding of biology. So that through our knowledge of the mechanisms of the binding of proteins, it will be easier for us to design drugs, especially drugs that target specific non-functional proteins.1 2

 Proteins have been used because they have many functions in the cell. A ligand has been used for its ability to bind to a protein with high affinity and specificity. Therefore, we will use the Bradford protein assay to know the properties of the binding between Ovalbumin and the Coomassie Brilliant blue dye, as this dye will change color over time. Also, this dye will show a change at the absorption of 596 nm, which indicates the binding to Ovalbumin.

The quantitative treatment of binding requires the development of an equation that contains all of the information necessary for understanding binding at the molecular level. So a simple interaction between a free ligand (L) and a free macromolecule (M) is described in equation: *(Protein–Ligand Binding Kinetics)*

L + M ↔LM (Eq.1)

 The affinity of the macromolecules with the bonding is described by a constant of formation (Kf), such that the macromolecule-ligand complex has Kf, and as shown in the equation:

Kf =([L][M])/([LM]) (Eq.2)

* **The Materials:**
* Ovalbumin solutions in water, two solutions: I = 5.0 mg/mL; II = 20 mg/mL
* Coomassie Brilliant Blue G-250 dye, stock solution, 9.0 x 10-4 M in ethanol, 85% phosphoric acid, H₂O.
* Dye solvent (100 ml ethanol, 200 ml 85% phosphoric acid, and 900 ml water)
* Automatic pipets and tips, 20 L, 200 μL, 1000μL
* 3-mL plastic cuvettes
* UV spectrophotometer
* 13 x 100 mm test tubes III.
* **Methods**:

**PART B + C: Binding of dye to oval albumin - saturation curve & Binding of dye to protein, the Scatchard plot:**

🡪 The spectrometer was turned on and the wavelength was set to 596 nm.

* Table\_1 was used to prepare the different solutions for absorbance measurements.
* All reagents have been near room temperature.
* Each solution has been prepared in a numbered test tube to correspond to table\_1.
* The dye solution was prepared by mixing 3.0 ml of dye stock solution with 27.0 ml of dye solvent.
* An automatic pipette system was used so that the appropriate amount of albumin oval II solution (20 mg/ml) was transferred into each tube.
* The total volume in each tube was adjusted to 3.0 mL by adding the appropriate amount of distilled water. Each tube was mixed well by holding a small square of hydrocarbon foil over the tube and turning it over several times.
* After about 15 min reaction time, the contents of each tube were transferred to a 3 ml cuvette after which the absorbance was read on A596 to the nearest 0.001 absorbance unit.
* A cuvette of distilled water was used as a blank.
* **Table\_1: Preparation of Cuvettes for Part B**

|  |  |  |
| --- | --- | --- |
| Tube | H2O | BSA(µl) |
| 1 | 750 | 250 |
| 2 | 748 | 250 |
| 3 | 746 | 250 |
| 4 | 744 | 250 |
| 5 | 742 | 250 |
| 6 | 740 | 250 |
| 7 | 735 | 250 |
| 8 | 730 | 250 |
| 9 | 725 | 250 |
| 10 | 700 | 250 |

* **Results:**

Figure 1: Saturation Curve of Coomassie Blue Dye binding to Ovalbumin protein with a Concentration of 20 mg/mL.

Figure\_2: The Scatchard Plot of Binding of Dye to Ovalbumin Protein at A596 nm.

* **Discussion:**

The free concentration of ligand molecules in solution is linked to binding in a quantitative analysis of a ligand-protein interaction.

As the figure\_1 shows to us, the curve is gradually increasing, and this indicates an increase in the binding sites with the protein, but the more the amount of protein we have, as we mentioned that the x axis represents the added concentration of the bonding molecules with the protein. The curve begins to become linear, which shows us that the binding sites have decreased, and the reason for this is that all the protein ligand complex have been bound, and thus the A max was determined to be equal to 0.772, which expresses the maximum binding point for the protein sites3.

The greater the bonding to the dye, the lower the absorption, and vice versa.

As it appears to us, there are two lines where each one expresses something different from the other. For example, when y = 0.0088x+0.1222, which is expressed by the red line, this indicates the amount of molecules that I have that have bound to the group of binding sites, and because these binding sites are very close and affinity to the ligand, this leads to a stronger bond than the part that is colored green and above It thus has fewer link sites and the points are farther from each other, indicating that it has a lower formation constant.

In order to produce this figure\_2, many calculations were used so that everything was related to a specific topic, for example, f was used to express the bound binding and [M]0 represents the initial concentration of the protein used while [L]0 represents the total initial concentration of the binding used as well.

So this equation was used:

([M]0)/f= 1/ (n Kf(1-f)) + ([L]0)/n

 So that in the end we can draw Figure\_2 above2.

* **Conclusion:**

 In conclusion, biochemistry is the study of chemical processes and pathways that occur inside the cell, proteins are one of the macromolecules that are important in the cell, so that a type of dye is associated with these proteins, which is Coomassi Brilliant Blue, which through the use of the spectrophotometer we can determine the regions of attachment in the protein.

* **References:**

 Du, X., Li, Y., Xia, Y.-L., Ai, S.-M., Liang, J., Sang, P., … Liu, S.-Q. (2016). Insights into Protein-Ligand Interactions: Mechanisms, Models, and Methods. International Journal of Molecular Sciences, 17(2), 144. DOI: 10.3390/ijms17020144

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3 Van Zoelen, E. J. J., Kramer, R. H., Van Reen, M. M. M., Veerkamp, J. H., & Ross, H. A. (1993). An exact general analysis of ligand binding displacement and saturation curves. *Biochemistry*, *32*(24), 6275-6280.‏

* **Appendix:**
* **PART C of figure\_2:**
* To find [M]0for each solution:
* *Tube 2's [M]0:*
* To calculate f for each solution:

f of tube 2 =

* to find from M1 V1 = M2V2
* y = 0.0088x + 0.1222
*
* To find from
* y = 0.0279x - 0.0674
* To find from

**Table\_2: The calculation of Scatchard plot**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Number of Tube** | **Abs** | **[M]0** | **f** | **1/(1-f)** | **[M]0/f** |
| **1** | 0 | 0 | 0 | 0 |  |
| **2** | 0.228 | 0.030 | 0.295 | 1.419 | 0.1018902 |
| **3** | 0.507 | 0.060 | 0.657 | 2.913 | 0.0916409 |
| **4** | 0.635 | 0.090 | 0.823 | 5.635 | 0.1097526 |
| **5** | 0.681 | 0.120 | 0.882 | 8.484 | 0.1364521 |
| **6** | 0.713 | 0.150 | 0.924 | 13.085 | 0.162910 |
| **7** | 0.72 | 0.226 | 0.933 | 14.846 | 0.2419893 |
| **8** | 0.726 | 0.301 | 0.940 | 16.783 | 0.3199858 |
| **9** | 0.74 | 0.376 | 0.959 | 24.125 | 0.392415 |
| **10** | 0.772 | 0.752 | 1.000 |  | 0 |

**Question:**

**🡪 Q.2 You are attempting to develop a colorimetric probe for use in binding studies. List several requirements that the probe must meet in order to be effective. For example, it must bind at specific locations of the macromolecule. Can you think of other requirements?**

1-It must bind at specific locations of the macromolecule (this has a binding site)

2- It must change color when it binds to a macromolecule.

3- The macromolecule-probe complex must be stable in order to avoid quick dissociation.

* **Q.3 A research project you are working on involves the study of sugar binding to human albumin. The sugars to be tested are not fluorescent, and you do not wish to use a secondary probe such as a dye. Human albumin has only one tryptophan residue, and you know that this amino acid is fluorescent. You find that the tryptophan fluorescence spectrum of human albumin undergoes changes when various sugars are added. Can you explain the results of this experiment and discuss the significance of the finding?**

The tryptophan in the active site of the protein is covered so that it can absorb a small amount and decreased of light so that this occurs upon binding. so resulting with a change in fluorescence properties

* **Q.4 Equation E5.5 in this experiment can be used to determine K, values, but hyperbolic plots are obtained. Convert Equation E5.5 into an equation that will yield a linear plot without going through all the changes necessary for the Scatchard equation:**

ṽ = ((n K*f* [L]) \ (K*f* [L] +1))

By calculating the reciprocal of both sides and using the right-hand shortcuts:

1\v= ((1\n K*f* [L] ) + (1\n))

A linear curve is generated by charting 1/v on the Y-axis and 1/[L] on the X-axis.