Cooperative Binding in Hemoglobin

Neil Ghugare

Assisting Mentor: **OBSCURED**

Abstract & Introduction

Hemoglobin proteins are found in the blood and are known for transporting oxygen. Each individual hemoglobin protein has four binding sites for oxygen. At any one time, a hemoglobin protein can have any permutation of oxygen molecules bound to it, including being empty (no oxygen molecules bound).

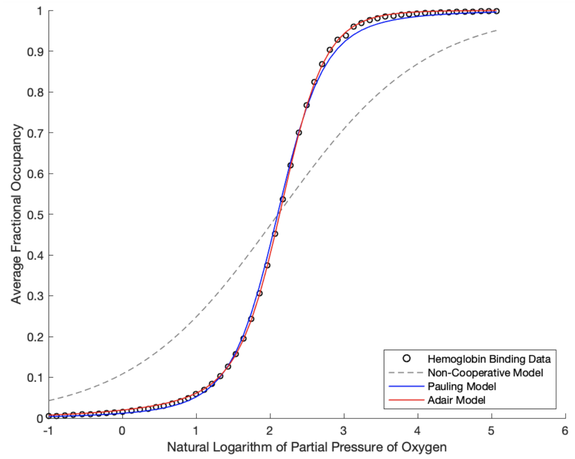

Figure 1. All Models on Hemoglobin Cooperation

By assuming non-cooperativity, pair cooperativity (Pauling), and full cooperativity (Adair), we fit the experimental data. We found the following mean absolute errors:

	Non-Cooperative	Pauling	Adair
Mean Absolute Error	0.1303	0.0077	0.0039

Table 1. Error Results of Non-Cooperative, Pauling, and Adair Models

The data used was collected from a paper by Kiyohiro Imai.[3]

The Biology

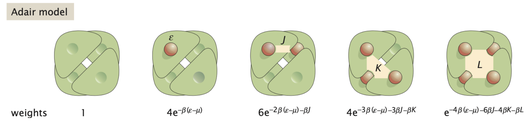

Figure 2. An Illustration of Hemoglobin Oxygen Binding Sites (Phillips, 2012, p. 302)[1]

The regions J, K, and L represent cooperativity between oxygen molecules. We can assume $J = K = L = 0$ for non-cooperativity and $K = L = 0$ for the Pauling model. This will later translate to $j = k = l = 1$ for non-cooperativity and $k = l = 1$ for the Pauling model for parameter fitting. We wish to understand if cooperativity exists between the oxygen molecules bound to hemoglobin.

Important Equations & Derivations

These equations were vital in modeling the experimental data. A link for derivations (done by hand) is found in the References section.[2]

1. **Grand Partition Function**. This function sums over many Boltzmann factors (the weights of each state). Once normalized, this function can help us calculate the average fractional occupancy.

$$\mathcal{Z} = \sum_s e^{-\beta(E(s)-\mu N_s)}$$

2. **System Energy**. This equation represents the energy of the system in the Pauling Model. There is another version with K and L for the Adair Model. For Non-Cooperativity, we assume $J = 0$.

$$E = \varepsilon \sum_{\alpha=1}^{4} \sigma_\alpha + \frac{J}{2} \sum_{(\alpha,\gamma)} \sigma_\alpha \sigma_\gamma$$

3. **Average Fractional Occupancy**. The average fractional occupancy of oxygen particles based on the above function, the chemical potential μ, and a constant β.

$$\langle N \rangle = \frac{1}{\beta} \frac{\partial}{\partial \mu} [\ln(\mathcal{Z})]$$

4. **Parameter Modeling**. This equation helps with the modeling of the non-cooperative and Pauling models. For the rest of this project, we used a fit parameter b instead of x related to the collected data of oxygen partial pressure.

$$\langle N \rangle = \frac{4x + 12x^2 j + 12x^3 j^3 k + 4x^4 j^6 k^4 l}{1 + 4x + 6x^2 j + 4x^3 j^3 k + x^4 j^6 k^4 l}$$

Brute-Force Error Searching

This is how we found low error approximations for b and j for the Pauling model. For the Non-Cooperative model, this method is simplified to a one-dimensional search over b.

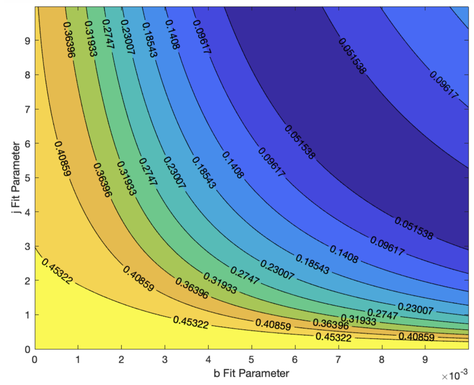

Figure 3. Contour Plot of Error for Pauling Model

Finding this band of lowest area was found manually by searching for the lowest error on a basic contour plot, and then changing the range of b and j as needed. After this point, a value of $b = 0.0100$ and $j = 5.2985$ was chosen to use in the Pauling model.

The Monte Carlo Simulation

Brute-Force parameter fitting for all four parameters (b, j, k, and l) for the Adair model is not only time consuming, but takes a lot of computing power. Instead, we employed a Monte Carlo simulation. The logic is as follows:

- Choose random values for b, j, k, and l (within a finite range)
- Calculate $\langle N \rangle$ and the error of the model using those values
- If the error is lower than the current lowest error, save the values and set the new error
- Iterate for many iterations (we used $300,000$)

After running this simulation many times, we got different model fits. All of the models, however, had a lower error than the Pauling model.

Iteration	1	2	3	4	5	Avg
Error	0.0033	0.0031	0.0031	0.0068	0.0033	0.0039

Table 2. Adair Model Errors through Monte Carlo Simulation Iterations

In general, it is possible to get a model that has a higher error than the Pauling model due to random chance. By using a large number of iterations (or even increasing the total amount) we can reduce the likelihood of that happening. Therefore, we generally get a model that has a lower error than the Pauling one.

Results & Conclusion

Through the analysis of the data, we found that the Adair model with full cooperativity had the lowest error compared to the experimental data of 0.0039. Then the Pauling model with cooperativity of oxygen molecule pairs had the next lowest error of 0.0077, and the non-cooperative model had the highest error of 0.1303. With this, we can conclude that hemoglobin oxygen particles are cooperative in some manner.

Future Work

Future work could include comparing the calculated experimental b, j, k, and l values to common literature and see if the modeling lines up.

Acknowledgements

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- Jupyter Notebook (Project Jupyter, 2014)
- MATLAB (C. Moler & MathWorks, ca. 1970)
- LaTeX (L. Lamport, 1984)

References

[1] Rob Phillips et al. *Physical Biology of the Cell*, chapter 7.2.4. Garland Science, 2012.

[2] Neil Ghugare, 2023. `https://github.com/RandomKiddo/PolarisResearchProject`.

[3] Kiyohiro Imai. Precision determination and adair scheme analysis of oxygen equilibrium curves of concentrated hemoglobin solution: A strict examination of adair constant evaluation methods. *Biophysical Chemistry*, 37(1):197–210, 1990.

[4] Daniel V. Schroeder. *An Introduction to Thermal Physics*. Addison Wesley Longman, 200.