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Glucagon Modeled as a Coarse-Grained Bead-String Polymer

Final Project

Summary

Glucagon and insulin play major roles in regulating blood glucose levels, and people with type 1 diabetes mellitus need exogenous sources of these hormones for survival. One major issue faced by glucagon pumps that automate these exogenous injections is the problem of stability, as glucagon rapidly forms precipitates upon standard clinical preparation which greatly decrease their shelf lives. To understand general trends that cause these effects and determine conditions under which glucagon can be stored in an aqueous solution, a molecular dynamics simulation was conducted to illustrate the properties of a glucagon molecule under varying pH and temperature conditions. Glucagon was modeled as a bead-string polymer of 29 coarse-grain units, with each unit representing a separate amino acid and thus having its individual parameters, including different radii and charge. The changes in pH was modeled as changes in the total charge of each amino acid unit based on the charge of its R-group(s), and an Anderson thermostat was utilized to model changes in temperature. The radius of gyration of the simulated glucagon molecule increases with the distance from neutral pH. A similar, but plateauing trend can be seen with increasing simulation temperature. These trends have comparable counterparts to experimental results found in literature.

Background

Glucagon is one of two key hormones in the human body that constitute the regulatory actions of pancreas in controlling the blood glucose levels. People with Type 1 diabetes mellitus (T1DM) have a near total loss in these regulatory actions caused by the destruction of pancreatic β-cells by their immune systems, leading to life-threatening situations if left untreated [1]. In 2002 alone, over 21 million people were suffering from diabetes, and over 220,000 deaths resulting from complications due to diabetic symptoms [2]. To remedy the plight of people with T1DM, exogenous injection of glucagon and its counterpart insulin in a computer-controlled artificial pancreas system (APS) has gained wide-ranging support among the field of diabetes within the past decade.

One of several challenges that currently face a full-scale application of an APS system is the stability of the hormones while in storage. As the ultimate goal of an APS system is designed to allow people with T1DM a normalcy in their lives comparable to the general population, the internalized storage of glucagon must also be able to last for sufficient length of time prior to replacement. However, glucagon forms fibrils and/or rapidly precipitates out in forms commonly configured for clinical applications, which limit its usefulness as a form of storage in an automated pump system within the APS [3]. Therefore, it is of great value to study the conformation of glucagon under different conditions for which they can be kept within a useable form with minimal conformation.

Simulation Methods

Glucagon is a polypeptide consisting of 29 amino acids and a molecular weight of 3483 daltons [4]. Due to its relatively complicated structure with a chemical formula of $C_{153}H_{225}N_{43}O_{49}S$, its

structure was instead simplified to a bead spring polymer consisting of 29 bonded particles, each with its own individual radii and charge. A diagram that illustrates the resulting structure is shown in **Figure 1** [4].

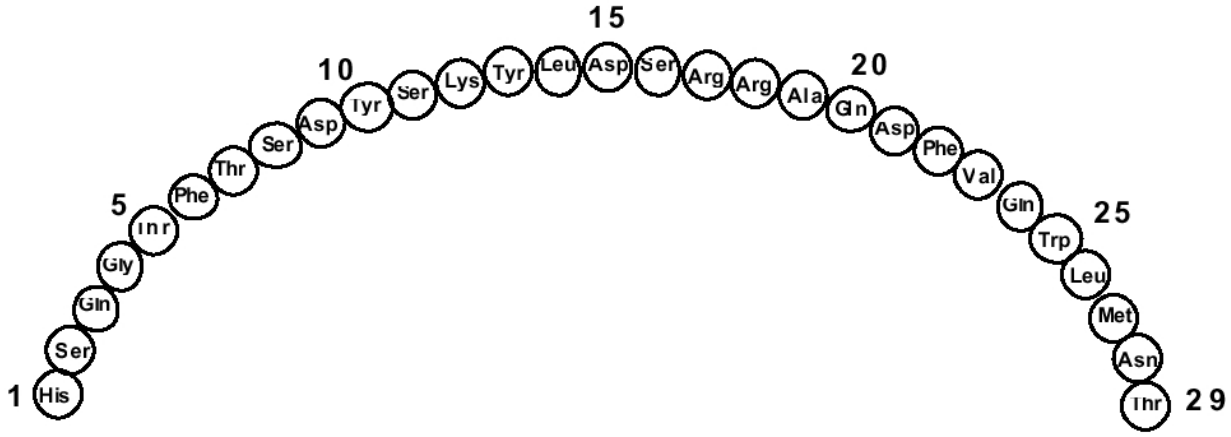

Figure 1: Bead-spring polymer model of glucagon that was used in the simulation [4].

Each amino acid in the molecule was considered a separate particle, and the interactions between the particles were modeled in three units. The electrostatic interactions between particles of different charges were modeled as a screened coulomb potential, as shown in **equation 1**. This is of great importance as amino acids contain carboxyl and amino groups whose charges vary with varying pH, which also means that the electrostatic interactions will greatly differ. The charges were signified as representations of their signs within the simulation code.

$$\phi(r) = \frac{Q}{4\pi\epsilon_0 r} e^{-k_0 r} \quad (1)$$

In addition to electrostatic interactions, the non-bonded interactions between the particles were simulated as Lennard-Jones potentials as shown in **equation 2**, and the bonded interactions were simulated as simple harmonic bond potentials as shown in **equation 3**.

$$V_{LJ} = 4\varepsilon \left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6}\right] = \varepsilon \left[\left(\frac{r_m}{r}\right)^{12} - 2\left(\frac{r_m}{r}\right)^{6}\right] \quad (2)$$

$$U_{bonded} = \frac{k}{2}(r - r_o)^2 \quad (3)$$

Basdevant et al published coarse-grain parameters of protein-protein potentials for each amino acid, and those parameters were used as the bases of each energy calculation [5]. Specifically, Basdevant developed a reduced protein model that represented each amino acid with different coarse grain levels. The center of each grain is placed in the geometric center of the heavy atoms, and their Van Der Waals potential minimum radii were derived through "a bottom-up procedure from the higher-resolution all-atom AMBER force field." [5] The specific values that were used for each amino acid are found in the included raw code of the simulation. The simulation was performed via the Virlet algorithm for molecular dynamics. The density of the glucagon model was fixed at 2e-6. The charge of each individual amino acid coarse grain was varied depending on their net charge under different pH conditions. The molecular simulation was conducted for

500000 steps, with the first 100000 steps used as equilibration. Δt of each step was set at 0.001. The dimensionless temperature was maintained at the target set point through an Anderson thermostat, and was varied from 1 to 4 to determine the effect of temperature on glucagon's stability. Each simulation was repeated five times to obtain the error. The radius of gyration of the model was averaged to determine the relative size of the model as a measure of the model's likelihood of coagulation.

Results and Interpretation

As **Figure 2** shows, the average radius of gyration of the simulated glucagon molecule increases at either extremes of the pH. This correlates with the literature data which mentions glucagon is soluble in aqueous solutions at pH less than 3 or greater than 9 [5]. One possible hypothesis for an explanation of this phenomenon is that at either extremes of the pH, the individual coarse grains carry a greater proportion of similar charges, which mean greater inter-molecular repulsion, and thus a greater radius of gyration. This leads to greater proportion of the polar R-groups within the molecule exposed to the solution, increasing its solubility in aqueous environments. On the other hand, a large proportion of the amino acids, represented as coarse grains in this simulation, carry net zero charge at neutral pH, leading to a smaller radius of gyration and thus a more "compact" molecule. This may lead to a lower polar characteristic of the overall molecule, which leads to a lower solubility.

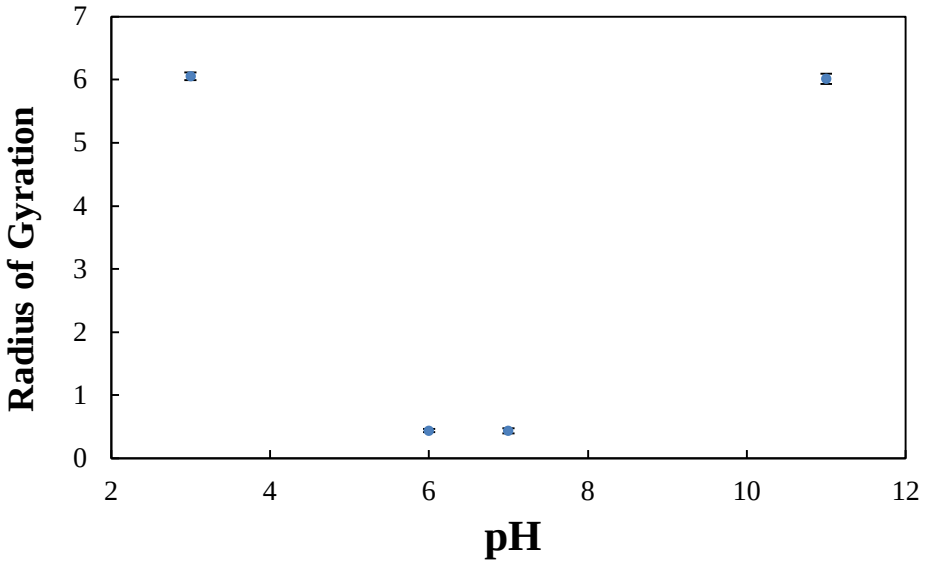

Figure 2: change in the radius of gyration of the glucagon model as a function of pH, with temperature fixed at 1.

Given the limitations of simplifying changes in pH only as changes in the charges of each carboxyl or amino groups, this is necessarily a highly limited approximation of reality. Further, the changes in the charges of the R-groups are highly delineated in specific pH, with the carboxyl groups losing the hydrogen at pH of around 3 (gaining a negative charge) and the amino groups losing the hydrogen at pH of around 10 (losing a positive charge). Histidine is unique in that the charge of its R group changes at or near physiological pH of 6.5. Thus, the modeling as done in this simulation would only have four discrete points – as shown in **figure 2**.

When the pH is fixed and the temperature set point in the Andersen thermostat is increased, the radius of gyration also increases correspondingly, as shown in **figure 3**.

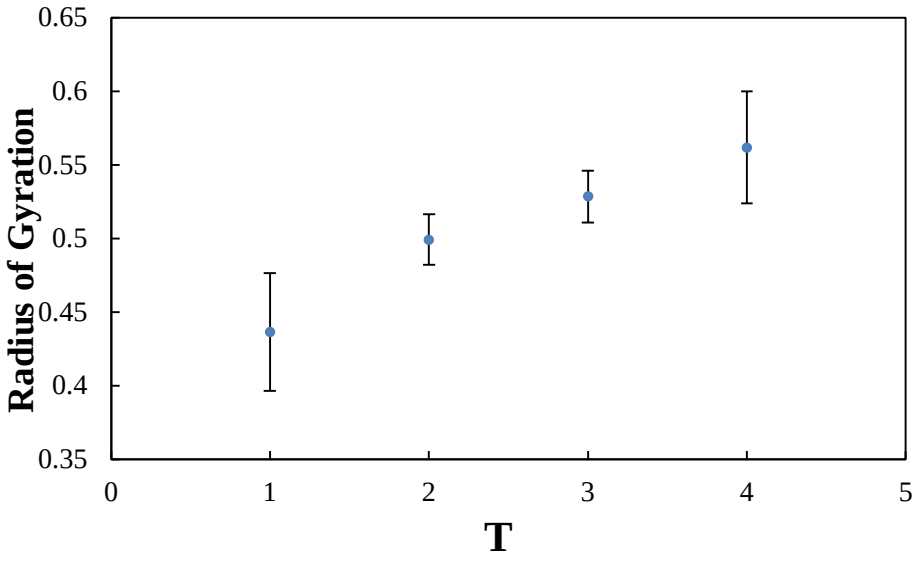

Figure 3: change in the radius of gyration of the glucagon model as a function of temperature, with pH fixed at 7.

As expected, given the same experimental conditions (apart from the temperature), the radius of gyration of the glucagon coarse-grain model increases with increasing temperature. This is expected considering the simulation parameters, which ignore reactions and simply focus on Van Der Waals, electrostatics, and bonding potentials. As temperature increases, the energy applied on each coarse-grain unit also increases, meaning that the velocity of each unit will also increase, leading to a general scaling up of the result of inter-molecular interactions and the possibility of greater solubility due to more polar interactions. However, once again this simulation disregards any additional reactions that may become significant upon increasing the temperature. Thus, a more intensive simulation of the complex in the presence of other particles must be conducted to fully understand the effect of temperature on glucagon stability – in fact, at near room temperature, glucagon appears to degenerate faster with increasing temperature [6]. This trend can also be interpreted from **figure 3**, as the increase in the radius of gyration appears to begin to plateau at T=4, meaning that the resulting increased solubility due to greater inter-molecular distances may be counteracted by reactions that promote precipitation as the temperature is further increased. Simply put, the molecule cannot "stretch" any further than its maximum length, but external reactions can be hastened at wider temperature scales.

There are multiple deficiencies in this model for which wide variety of improvements can be made. To begin with, this model is a coarse-grain representation of the actual molecule, and thus ignores highly important inter-atomic interactions between the various functional groups within each amino acid. As the amino acids are actually composed of charged R-groups and neutral groups, representing them as a sphere that encompasses the entire amino acid with a charge distributed throughout its entirety can be considered an oversimplification. Furthermore, only the harmonic bonding potential were taken into account in this simulation, and other bonding potentials, such as angular and torsional potentials, must be taken into account for a more accurate representation of the molecule. Moreover, this simulation is conducted on one glucagon molecule. As precipitation is necessarily a coagulation of multiple molecules, a more accurate representation of the effect of varying parameters on stability of a glucagon solution can be done my increasing the number of glucagon units to be simulated.

References

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