ENGINEERED DESIGN OF POLYMERS FOR NEUTRALIZING THE EFFECTS OF THE ANTICOAGULANT FONDAPARINUX

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INTRODUCTION

The most commonly used anticoagulants are heparin derived drugs [1]. Heparin derivatives include unfractionated heparing (UFH), low molecular weight heparins (LMWHs), and the synthetic pentasaccharide derivatives fondaparinux and idraparinux [2–5]. LMWHs are modified UFH with shorter chain length, more predictable pharmacokinetics and dose-response, and improved bioavailability [2, 6]. Fondaparinux and idraparinux are synthetic compounds that have predictable dose responses and an almost complete bioavailability [2, 5]. UFH and heparin derivatives are used for the treatment of serious illnesses including venous thromboembolism (VTE), unstable angina, and acute myocardial infarction [7–9]. They are also used in patients who are undergoing cardiac surgery and kidney dialysis [1].

While anticoagulation therapy is widely used, it has certain undesirable side effects such as excessive bleeding. In the event of an overdose of anticoagulants, antidotes should be administered to neutralize the anticoagulants and prevent bleeding complications while still avoiding thrombosis [10, 11]. New anticoagulants are regularly being created and therefore the development of effective antidotes with minimal side effects is a subject of major interest in the therapeutic field [10, 12, 13]. For instance, the synthetic anticoagulant fondaparinux has become increasingly important in clinical medicine because of its advantages over UFH and LMWHs; however, it does not yet have a specific antidote [3, 5, 10, 12, 14]. Therefore, the development of a clinically safe antidote for this anticoagulant is critical [13].

Drug discovery is a very difficult, labour-intensive, expensive, and time-consuming process. On average, each drug that came onto the market in the 1990s cost over US$800 million and took 14 years to be developed [15]. This is due to an optimization process that typically requires the synthesis of hundreds or thousands of new molecules. The use of computer-based techniques however, can speed up this process [16]. The Department of Pathology and Laboratory Medicine at the University of British Columbia is currently developing a promising polymer based antidote for fondaparinux. However, the structural design of this polymer antidote must be modified to bind fondaparinux with high affinity thus effectively neutralizing it. Therefore, the overall goal is to improve the design process by using computer simulations to develop a synthetic polymer that can electrostatically neutralize fondaparinux. The objective of this work is to implement a mathematical model for the characterization of binding between fondaparinux and a polymer antidote.

MATERIALS AND METHODS

Promising Antidote for Fondaparinux

Fondaparinux does not currently have a specific antidote [3, 5, 10, 12, 14]. A promising polymer based antidote for it is currently being developed by the Department of Pathology and Laboratory Medicine at the University of British Columbia. The cationic binding unit within the polymer antidote is tris[2-(dimethylamino)ethyl]amine, which has a formal charge of +4 [17]. These units are attached to the polymer antidote's core and are randomly distributed on its surface (Figure 1). While the current polymer antidote designs effectively neutralize UFH and LMWHs they fail to neutralize fondaparinux [17]. However, it is believed that the binding affinity of the polymer antidote and fondaparinux can be manipulated by modifying the former's charge density [17]. An effective polymer antidote will bind fondaparinux with high affinity, neutralizing it.

Electrostatic Interactions Model for Fondaparinux and Its Polymer Based Antidote

Given the large number of possible structural configurations for the polymer antidote, an experimental approach to find its structural design that will provide the most stable complex with fondaparinux will be expensive and time con-

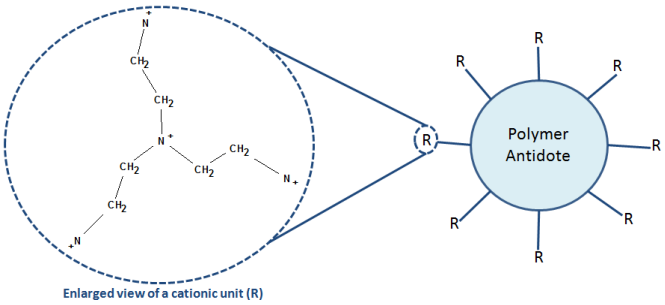

Figure 1: Schematic representation of fondaparinux polymer antidote. The cationic binding unit (R) on the polymer antidote is tris[2-(dimethylamino)ethyl]amine, which has a +4 charge. These binding units are attached to the core of the polymer and are randomly distributed on its surface.

suming. To reduce these constraints a mathematical model that will allow for the determination of the binding affinity between a candidate polymer antidote and fondaparinux is desirable. Specifically the association rate constant will be used as a metric for binding affinity and will thus need to be determined by the model:

$$\ln k_a = \ln k_a^o - \frac{\Delta U}{k_B T}\left(\frac{1}{1+ka}\right) \tag{1}$$

where k_a and k_a^o are the association rate constants in the presence and absence of long-range electrostatic forces, respectively; ΔU is the electrostatic energy of interaction; k_B is the Boltzmann constant; T is the temperature of the solution; a is the minimal distance of approach between the proteins; and k is the Debye-Hückel parameter. The Debye-Hückel parameter is defined as $k = \sqrt{\frac{2F^2 I}{\epsilon_o \epsilon_r RT}}$ [18], here, F is the Faraday constant, I is the ionic strength of the solution, ϵ_o is the the vacuum permittivity, ϵ_r is the dielectric constant of the solution, and R is the gas constant. The electrostatic interaction energy is defined as [19, 20]

$$\Delta U = U_{complex} - U_{moleculeA} - U_{moleculeB} \tag{2}$$

where U, the Debye-Hückel energy of a molecule, can be calculated from

$$U = \frac{1}{2}\sum_{i,j}\frac{q_i q_j}{4\pi\epsilon_o\epsilon_r r_{ij}}\frac{e^{-k(r_{ij}-a)}}{1+ka} \tag{3}$$

In this equation q_i and q_j are the charged atoms in the molecules and r is the distance between the charges.

Based on the Smoluchowski limit for the diffusion-controlled association of two uniformly reactive molecules where one is a small, rod-like molecule (i.e. fondaparinux) and the other is a large, spherical molecule (i.e. polymer antidote), k_a^o can be calculated as [21]

$$k_a^o = 4\pi N_A (D_A + D_B) R' \tag{4}$$

here, N_A is Avogadro constant, D_A and D_B are the diffusion constants of molecules A and B, respectively, and R' is the interaction radius. R' is defined as $\frac{l}{ln(2l/w)}$, where l and w are the major and minor semi-axes of the ellipsoid. The diffusion constants are calculated as

$$D_A = \frac{k_B T}{6\pi\eta r_A^{'}} \quad \text{and} \quad D_B = \frac{k_B T}{6\pi\eta r_B^{'}} \tag{5}$$

where, $r_A^{'}$ and $r_B^{'}$ are the hydrodynamic radii and η is the viscosity of the solvent.

Schreiber and co-workers used equations 1 to 3 to enhance the rate of association above a certain basal rate (i.e. rate of association in the absence of electrostatic forces) by improving the Coulombic complementary between the charged residues on the surface of the proteins [19, 20]. Similar to protein-protein interactions it has been demonstrated that the cationic segments of a polymer similar to the polymer antidote interact electrostatically with the negative charges of bio-macromolecules [17, 22] and, therefore, form stable complexes [22]. Also, it has been shown that the binding between antithrombin and the heparin pentasaccharide unit structure responsible for binding to antithrombin (almost identical sequence of sugars as those found in fondaparinux [2, 4, 13]) was mediated primarily through electrostatic contacts [23]. Therefore, it is clear that electrostatic interactions play a major role in the formation of the polymer antidote-fondaparinux complex. It is thus the approach of this work to use the empirically proven Schreiber's model to determine k_a between the polymer antidote and fondaparinux.

RESULTS

Binding of Fondaparinux

The first step to characterize the binding between fondaparinux and its polymer based antidote was to determine the number of binding units (N) on the polymer antidote that are required to bind one fondaparinux molecule. Fondaparinux is a pentasaccharide unit with 10 anionic sites, each baring a formal charge of -1. The length of fondaparinux was determined to be 2.52x10^{-9}m by using the three-dimensional chemical structure viewer Jmol . As previously mentioned, each cationic binding unit on the polymer antidote has a formal charge of +4. Therefore, based on the size of fondaparinux and the respective number of charges of fondaparinux and a cationic binding unit, computer simulations were performed to test two possible binding cases: a) fondaparinux will bind preferentially to groups of three binding units but also to any closely spaced pairs and b) fondaparinux will only bind to groups of three binding units. This was performed for various polymer antidotes that differed in the number of binding units. Based on an

isothermal titration calorimetry (ITC) experimental result it was determined that three binding units are required to bind a fondaparinux molecule (Figure 2).

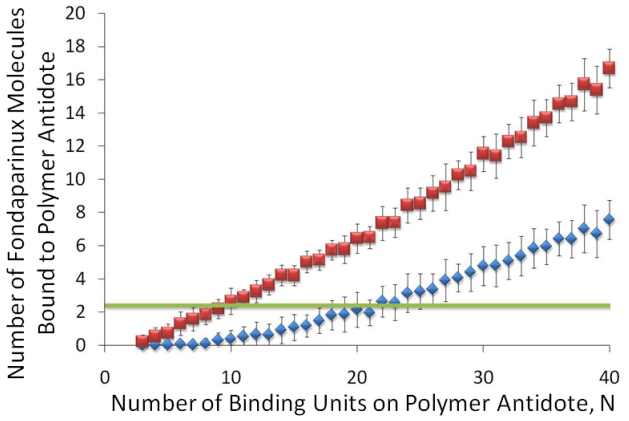

Figure 2: Number of fondaparinux molecules bound to polymer antidote when binding requires: 2 or 3 closely spaced binding units (square) and 3 closely spaced binding units (diamond). Experimental value (line) for an undisclosed N (due to proprietary reasons). The error bars represent the standard deviation of 50 calculated values.

Sensitivity Analysis of a

Once the number of binding units required to bind a fondaparinux molecule was determined, k_a was calculated for polymer antidotes containing up to 40 binding units randomly spaced on the polymer's surface. These computer simulated values were found to be unrealistically high (1x10^{200}) indicating that a minimal distance between binding units, $dmin$, was required. A $dmin$ of 1x10^{-9}m was chosen as it is the maximum value that allows for 40 binding units on the surface of the polymer antidotes under study. Also, it is less than half of the length of a fondaparinux molecule allowing fondaparinux to still bind to three closely binding units. Using a $dmin$ of 1x10^{-9}m, k_a values were again calculated. Similarly to the cases where $dmin$ was less than 1x10^{-9}m, the k_a values continued to be unrealistically high. Therefore, a sensitivity analysis was performed on a polymer antidote with 18 binding units to determine an appropriate value for a. The results show that a=1x10^{-9}m generates values of k_a closer to those previously reported [25] (Figure 3).

Computer Simulated k_a for Polymer Antidote-Fondaparinux Binding

After determining an appropriate value for a, k_a was again calculated for polymer antidotes that differed in the number of binding units (Figure 4). For up to 20 binding units k_a results were in the expected range. However, for N>20 k_a values were unrealistically high. This indicates

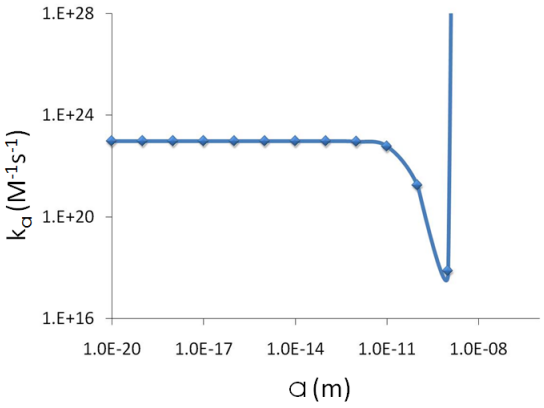

Figure 3: Effect of minimal distance of approach (a) on computer simulated k_a values. Polymer antidote contained 18 binding units randomly spaced no closer than 1x10^{-9}m.

that $dmin$ is likely greater than 1x10^{-9}m and that a larger value of a is required. In turn this suggests that the binding units must be flexible in order to still allow for the binding of fondaparinux which has a length of only 2.52x10^{-9}m and possibly precludes the possibility of placing 40 binding units on the surface of polymer antidotes with a radius of 3.8x10^{-9}m. Further study is required.

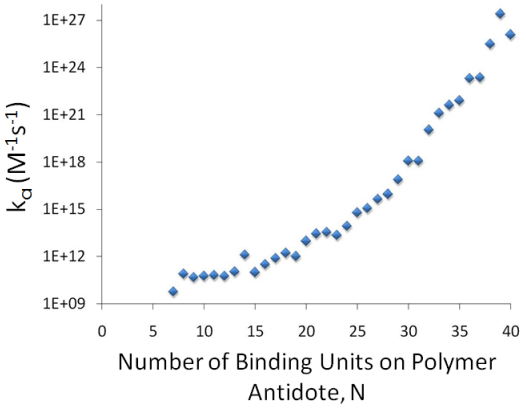

Figure 4: Computer simulated k_a for the binding of fondaparinux to polymer antidotes that differ in the number of binding units.

CONCLUSIONS AND FUTURE WORK

A mathematical model that uses a relationship between the electrostatic energy of interaction and the rate enhancement of the formation of a complex was used to gain knowledge on the polymer antidote-fondaparinux binding. By comparing computer simulated values with experimental results, it was determined that three binding

units are required to bind a fondaparinux molecule to a polymer antidote. After performing a sensitivity analysis on a and calculating k_a values for polymer antidotes that differed in the number of binding units, it was found that a minimal distance between the binding units that is greater than $1\text{x}10^{-9}$m must be implemented. This means that binding units are most likely flexible to allow for binding of fondaparinux and further study is required. Also, experiments must be performed for model validation. These will characterize the electrostatic charge neutralization of fondaparinux by the polymers as well as determine the binding strength and the association and dissociation rate constants.

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