A MODEL OF BINDING OF DOXORUBICIN WITH HEPARIN AND ENOXAPARIN

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Abstract: *Chemotherapy is one of the most successful methods of fighting with cancer, but like almost any kind of therapy, it has disadvantages such as severe side effects due to the high dose and/or unrestricted distribution within all body compartments. There have been numerous attempts to overcome these difficulties. The first option is to create a new active compound, what has shown to be very costly and, in many cases, ineffective. The other solution is to develop a new way to transport and release of existing drug in the organism using targeted drug delivery systems. Doxorubicin has been selected as a cytostatic substance that are already approved for medical use. The strategy is based on two features; entrapment of the active ingredient within the carrier by associating it with polymer and release it using external trigger guided by an imaging technique. The doxorubicin can be associated with heparin or enoxaparin. In order to implement the heparin/doxorubicin complex into the carrier structure, the thermodynamics of the aggregate formation need to be quantitatively described. The isothermal titration calorimetry has been used to extract the quantitative measures of its formation and stability, necessary for the designing both; pharmacological strategy and production process. To this end, the theoretical model of the binding has been developed and tested on well-defined experimental systems using heparins with different polymer lengths.*

Keywords: HEPARIN, DOXORUBICIN, ITC, BINDING MODEL

1. Introduction

Chemotherapy is the well-established pharmacological approach in the treatment of cancer. Despite unquestionable successes application of chemotherapy carries serious risks of potentially dangerous side effects, which imposes a limitation on the drug dose. One of the ongoing efforts is focused on the designing pharmacological strategy, which is based on supramolecular devices [1,2]. The strategy utilizes a well-established compound as an element of the device design to deliver and release it at the selected locations within the body[3]. The main objective of our ongoing research is the construction of a new kind of carrier that will be injected to a patient body and a drug released by an external trigger such as ultrasounds. This approach will allow for a precise release of drugs at tumor location. The design of the effective release mechanism requires that the encapsulated drug is associated with a compound which will prevent its uncontrolled release.

For these purposes, the suitable polymer for the drug association needs to be selected and thoroughly described. At the first step, the binding stoichiometry will be determined. Next, the effect of different solvents on the aggregate stability will be evaluated. To this end, the calorimetric experiments have been performed and obtained thermograms analyzed.

For purpose of this research, the doxorubicin has been selected as an active compound and heparin and enoxaparin as polymer scaffolds for doxorubicin association. Doxorubicin is a substance that is widely used in oncology [4,5,6], hence its application as an active ingredient has already been approved by authorities.

2. Measurements

In order to collect the high-quality experimental data for model fitting, we test the variety of experimental arrangements. Base on these studies doxorubicin at concentration 1.84 mM as titrant was selected. As substance in the cell, we use 0.045 mg/ml of both heparin and enoxaparin dissolved in 10 mM HEPES. The doxorubicin and heparin solutions were prepared before each experiment. Before use, every solution was degassed for 30 min with mixing at temperature 22 ^{o}C. All measurements were performed using ITC calorimeter (TA Instruments NanoITC 2G). The volume of the sample cell and the titrating syringe were equal to 1019 µl and 250 µl respectively. Each experiment consisted of 25 injections of 10 µl volumes, except the first one, which was equal 1.14 µl and discarded in the subsequent analysis. Times between injections were set to be 300 s. All measurements were performed at 22 ^{o}C.

Results

Example of thermograms, obtained in the experiment when enoxaparin was titrated with doxorubicin, are shown in Figure 1, whereas the same experiment performed with heparin is shown in Figure 2. Left panel shows row experimental data, whereas right panel the same data corrected for the baseline. When 0.045 mg/mL heparin was titrated with 1.84 mM doxorubicin similar but not identical thermograms were collected (Figure 2).

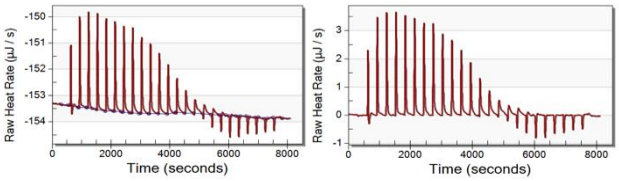


Figure 1. Example of thermograms obtained when 0,045 mg/mL of enoxaparin dissolved in 10 mM HEPES buffer (pH = 7.4) was titrated

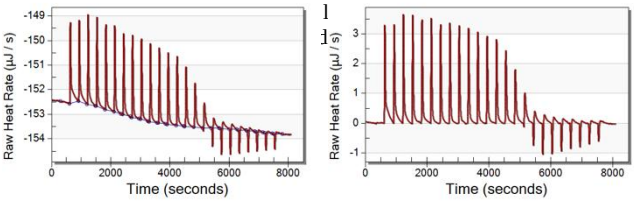


Figure 2. Example of thermograms obtained when 0.045 mg/mL of heparin dissolved in 10 mM HEPES buffer (pH = 7.4) was titrated with 1.84 mM doxorubicin. The left panel shows row experimental data, whereas right panel the same data after baseline subtraction.

Quantities of heat flow in titration experiments were appropriately corrected for the heat of dilution. Example of such data is presented in Figure 3, where doxorubicin was titrated into

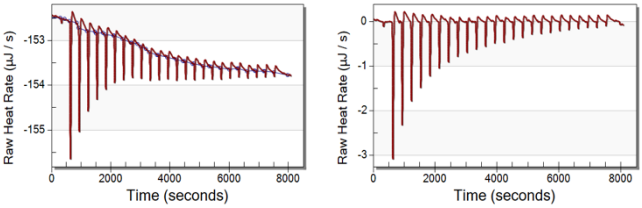


Figure 3 Example of thermograms obtained when 10 mM HEPES buffer (pH = 7.4) was titrated with 1.84 mM doxorubicin. Left panel shows row experimental data, whereas the right panel the same data after baseline subtraction.

the 10 mM HEPES buffer alone.

3. The model

The doxorubicin-polymer binding process was approximating with the binding model, in which binding seats are independent and ligands are not interacting with each other. The binding is mainly driven by electrostatic interaction, which is described by the Stern model since doxorubicin and polymers are charged (Figure 5 and Figure 4).

Figure 1 Single heparin monomer[7].

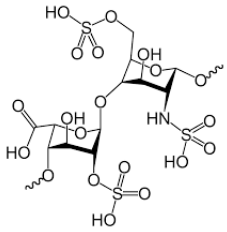
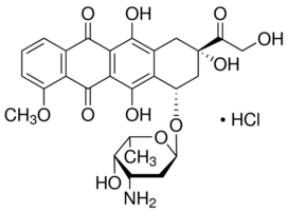


Figure 5 Chemical structure of doxorubicin[8].



4. Model fitting to experimental data

All fitting procedures were done using NanoAnalyze v.3.8.0 software made by TA Instruments. Experimental data was fitted to the equation 3, that is a one of model contained in software that we use. Fitting quality was assessed on basis of Monte Carlo simulation checking the susceptibility of the model to random disturbances. The average deviation of ΔH was determined to be about 5 % of the determined value and the n deviation was between 2 and 5 %.

$$Q = \Delta H * 10^{-9} * \left(L_{Bound\ ,i} - \frac{L_{Bound\ ,i-1} * (V_t - V_i)}{V_t}\right) \quad (1)$$

Where V_t – total volume, V_i – volume of ith injection.

$$L_{Bound\ ,i} = \frac{a - \sqrt{(a - 1019 * 10^{-6})^2 - 4\left(\frac{1}{K_d}\right)^2 L_i T_i n}}{2\frac{1}{K_d}} \quad (2)$$

Where K_d - L_i - concentration of ligand after ith injection, T_i - concentration of cell after i-injection, n – stochiometric number.

$$a = -\frac{1}{K_d}(L_i - T_i n) \quad (3)$$

Where K_d - L_i - concentration of ligand after i-injection, T_i - concentration of cell after ith injection, n – stochiometric number.

The heat flow after each injection was calculated as an area under the peak calculated from thermograms.

Examples of experimental data along with fitted functions, for bot enoxaparin and heparin, are presented in Figure 5 and Figure 6. In both cases the difference between heats of binding and dilution are fitted.

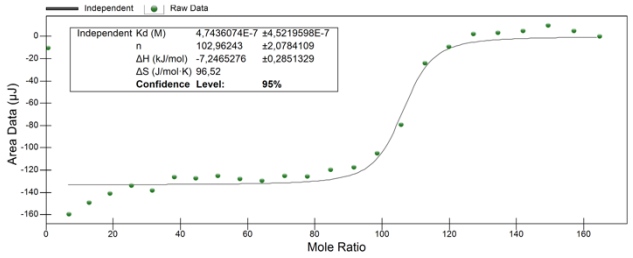


Figure 6 Example of fitting the heat flow generated by interaction between doxorubicin and heparin with the independent binding seat model.

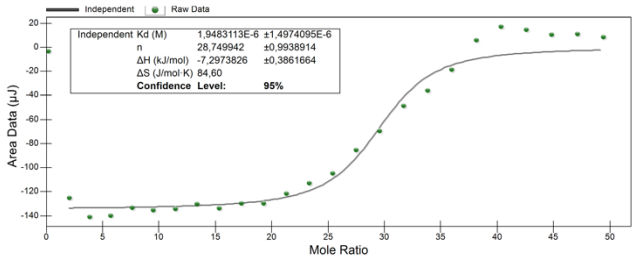


Figure 7 Example of fitting the heat flow generated by interaction between doxorubicin and enoxaparin with the independent binding seat model.

The average value for results of fitting experimental data determined for both polymers is presented in Table 1. Association of heparin with doxorubicin is accompanied by larger enthalpy change than that obtained for enoxaparin indicating stronger binding. This is accompanied by the large difference in a number of binding seats per heparin molecule and smaller dissociation constant.

Table 1 The average value of parameters retrieving from fitting of experimental data to the model.

Symbol	K_d	n	ΔH
Unit	M	-	kJ/mol
Heparin	4,50E-07	98,68	-7,84
Enoxaparin	1,74E-06	28,44	-6,55

5. Conclusion

Calorimetric data shows that the doxorubicin interacts with negatively charged polymers and that the interaction depends on the size (number of binding seats) on a polymer. Specifically, doxorubicin binds to heparin almost an order of magnitude stronger than with smaller enoxaparin. In addition, there are three times less binding seats on enoxaparin than heparin. The determined quantities are critical for the design of a carrier with tuneable parameters, which are important for an aggregate stability.

6. Acknowledgments

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7. References

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