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Fluorescently labeled bovine RNase and bovine lactalbumin interactions with anti-fluorescein antibody

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Nicole Newman, Allison Grodnick, Henry Jakubowski

Introduction

Antibodies are important immune system proteins, which binds to foreign molecules called antigens. This signals other immune molecules and cells into action.

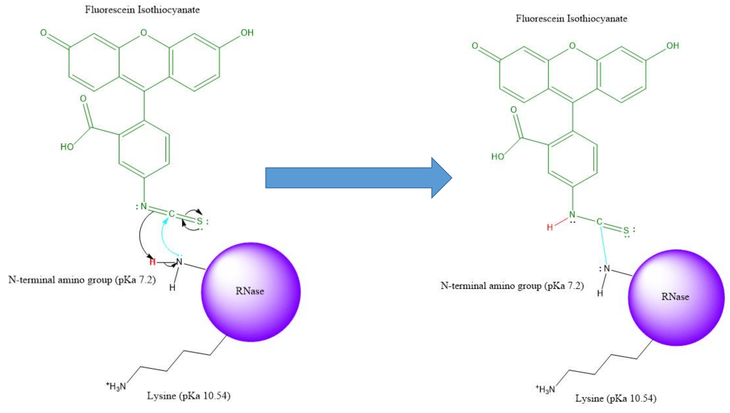
Antibodies interact very specifically with antigens. Less is known about how tightly antibodies and antigens interact. These interactions can be explored by using a model system of a monoclonal antibody recognizing a fluorescein epitope on fluorescein-labeled proteins.

The focus of this research is to develop methods to detect binding of fluorescein-labeled RNase or Lactalbumin antigens to a monoclonal antibody to fluorescein. Two methods will be used: fluorescent spectroscopy and an enzyme-linked immunosorbent assay (ELISA).

The antigens are fluorescently modified preferentially on the N-terminal amino group. This single site specificity at the epitope of the antigen makes it easier to observe the interactions of the antibody-antigen complex. The goal of this research is to create a model system of antibody-antigen interactions.

Methods

- Bovine RNase (5 mL, 1mg/mL) in PBS buffer (5 mM sodium phosphate/0.1 M NaCl, pH 7.5) was mixed with FITC (13 mL, 105 ug/uL in DMF), a molar ratio of 1:1.5 of protein to fluorescein
- Labeled RNase and excess free FITC were separated with size exclusion chromatography (G25/ PBS buffer (5 mM sodium phosphate/0.1 M NaCl, pH 7.5))
- Fluorescent excitation and emission spectrums were taken using a spectrofluorometer to characterize the modified- spectrum indicated modification and to measure antigen-antibody interactions



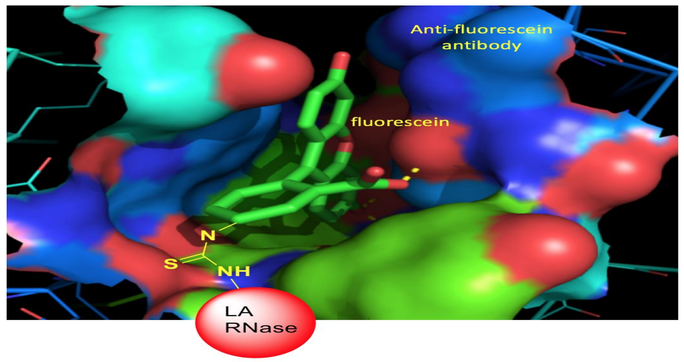


Figure 5. Covalent binding of modified RNase with FITC to anti-fluorescein antibody.

Results

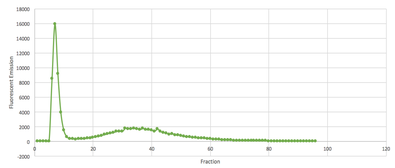


Figure 1. Size exclusion chromatography of fluorescein-labeled RNase as measured by fluorescence emission (λ excitation = 482 nm, λ emission = 490-510 nm). Peak 1 is modified protein and peak 2 is excess FITC.

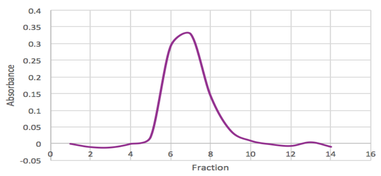


Figure 2. Size exclusion chromatography of fluorescein-labeled RNase as measure by absorbance at 280 nm (same fractions as in Figure 1).

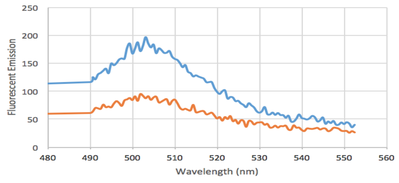


Figure 3. Comparison of emission spectrum in PBS buffer solution (pH 7.5,) λ excitation = 482 nm

- RNase + anti-fluorescent antibody (orange)
- RNase - anti-fluorescent antibody (blue)

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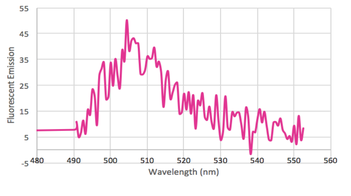

Figure 4. Difference emission spectrum (fluorescence intensity without antibody – fluorescence intensity + antibody) for Figure 3 showing quenching of fluorescein fluorescence by anti-fluorescein antibody.

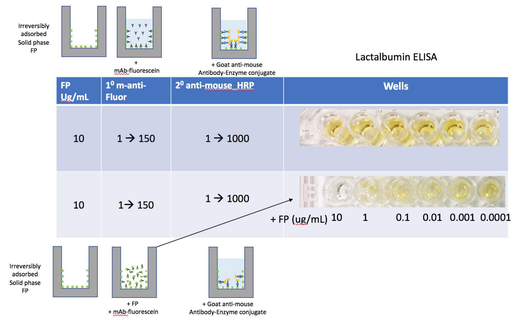

Figure 5. ELISA assay results of bovine lactalbumin with varying concentrations of fluorescently labeled bovine lactalbumin (row 2) compared to control group without fluorescently labeled bovine lactalbumin (row 1).

Discussion

- RNase and lactalbumin were labeled with a 1.5:1 stoichiometry of fluorophore to protein to modify the N-terminal amino group. Excess fluorophore was removed via size exclusion.
- The fluorescent peaks in Figure 1 show the excess fluorophore (peak 2) and fluorescently modified antigen (peak 1). Figure 2 shows that first fluorescent peak in Figure 1 is protein. Results indicate a 1:1 of fluorophore to antigen.
- Figure 3 shows that the antibody quenches the fluorescence of the fluorescein on the labeled protein, indicating binding to the fluorescein. This can be readily seen in Figure 4.
- Our data suggests that there was antibody-antigen interactions, but the peak does not level out from saturation, so Kd cannot be found.
- The ELISA assay results show that as the concentration of free fluorescein-labeled antigen in solution increases, the amount of primary antibody binding to the free antigen increases. This in turn decreases the primary antibody binding to the bound antigen in the plate. This results in a less intense color in the assay.

Future Plans

- The next step of the experiment is finding the dissociation constant (K_D) by varying the concentration of antibody.
- Complete and analyze an ELISA assay.
- Exploring other methods of calculating the K_D.

References

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Acknowledgments

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