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Enzyme conjugation for enhanced adsorption

Students as Partners

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1 Abstract

The goal of this project was to conjugate lysozyme and alginate using a process called the Maillard reaction. It is to serve as a proof of principle and a spring board for future work. The results of this experiment suggest that the alginate has been successfully bound to the lysozyme, though the results might be heavily affected by errors in the correction for differing lysozyme-concentrations in the samples.

During the project, I have gotten a lot of experience working in the lab, with the work ranging from mixing of chemicals to doing measurements using UV-Vis and fluorescence spectroscopy. I would suggest a similar project to anyone who has plans of working in the lab in the future.

2 Introduction

Biocatalysis has emerged as an important technology in green and sustainable synthesis of pharmaceuticals, vitamins, flavor and fragrances. The goal of this project was to conjugate an enzyme and carbohydrates, and would serve as a proof of principle. This was done by conjugating the enzyme lysozyme and the negatively charged carbohydrate alginate via a process called Maillard reaction. This reaction is a process in which the reactive carbonyl group of the carbohydrate and the nucleophilic amino group of the amino acid are being bound. This Maillard reaction, or glycosylation of the enzyme, has shown to greatly improve the stability of the enzyme [1] as well as having excellent emulsifying and antimicrobial effect [2].

3 Method

This project used wet lab techniques, i.e. most of the experimental parts were done in the lab, mixing various types of chemicals. Stock solutions of lysozyme and alginate were prepared, before mixing them at different ratios and incubating them at a specific temperature and relative humidity for different durations. After mixing the chemicals and completing the glycosylation reaction, different tests were conducted to asses the results. Two different methods were used to acquire the concentrations of protein in the conjugate enzyme-carbohydrate mix. Firstly, UV-Vis spectroscopy was used to determine the concentration of lysozyme in the samples by relating the measured absorbance to the concentration using Beer-Lambert's law. The other method used to determine the concentration of lysozyme was using fluorescence spectroscopy. A standard curve was made with known concentrations of lysozyme, and the concentrations in the glycated samples were acquired from this. A fluorescamine assay was used to assess how many of the carbohydrate tails had bound to the lysozymes by measuring the fluorescence intensity.

4 Results and discussion

After doing the UV-Vis experiment, it was evident that the concentration could not be determined from this method. This was due to the alginate also having high absorption at the peak wavelength for lysozyme. Therefore it was not possible to determine the absorbance of the lysozyme in the solutions, and consequently the concentration of lysozyme.

However, this was possible using fluorescence spectroscopy. By inspecting the control samples in Figure 1, one sees that the two negative controls (samples with alginate and with only buffer) were negligible compared to the fluorescence intensities from the lysozyme in the samples.

Still, correcting for the differing concentrations in the samples proved to be difficult using these values, so a different method was chosen. The theoretically correct concentrations of the samples were calculated retrospectively at each step of the process, and the resulting concentration was used as a corrective factor. However, there are significant errors in this method due to carelessness during the weighing process. The fluorescence intensities of the samples are shown in Figure 2.

As can be seen from these results, there is a large variation in fluorescence intensities. Lower fluorescence intensities suggests that fewer primary amine groups are available to fluoresce due to them being bound to carbohydrate tails. Conversely, the higher fluorescence intensities suggests that these samples have not had carbohydrate tails bound to the amine groups of their lysozymes and thus they fluoresce more. However, due to the large uncertainty in the correction for differing concentrations of lysozyme in the samples, some of the results may be skewed.

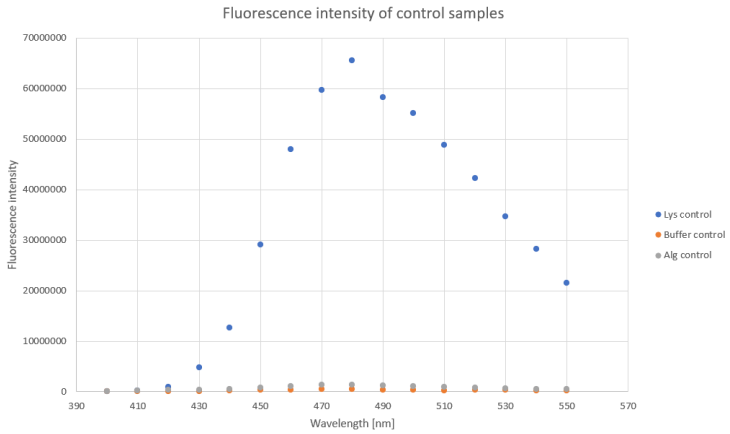

Figure 1: The fluorescence intensity of the control samples at different wavelengths. The positive control (Lys control) contains fluorescamine and lysozyme in buffer. The two negative controls contain fluorescamine and alginate in buffer (Alg control), and fluorescamine and buffer (buffer control).

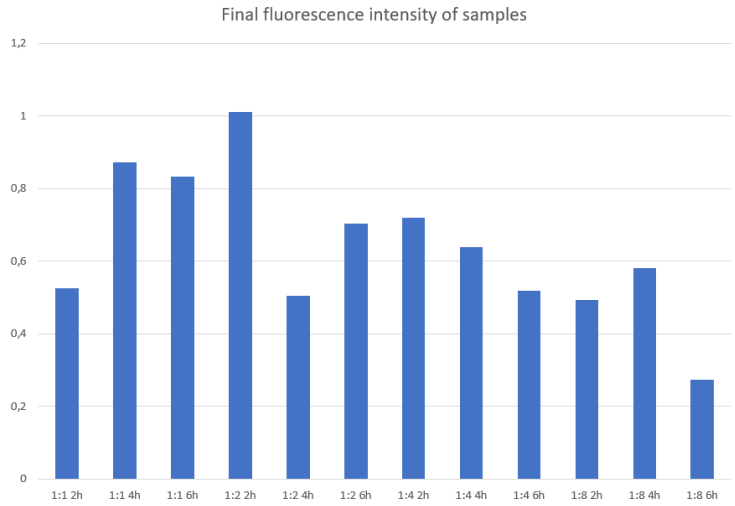

Figure 2: The final fluorescence intensities of the samples after correction for concentration-differences. Four different ratios of Lys:Alg (1:1, 1:2, 1:4, 1:8) were incubated at three different durations (2h, 4h, 6h). All intensities are relative to the intensity of the positive control.

However, these results do suggest that having higher ratios of lysozyme to alginate might result in more bindings, since the intensities are lower for higher ratios of Lys:Alg. Also, it might seem like the glycosylation process is improved

by having the samples incubating over a longer period, since the trend is that the fluorescence intensity drops for these samples. From these results, it would seem like alginate has indeed been bound to the primary amine groups of the lysozyme, and that the results are better for the longer incubation periods.

5 Experiences

I have gotten a lot of experience working in wet lab during the course of this project. As a physics student I have had courses that give some experience in the lab, but I found them to be insufficient to prepare one for future work in the lab. Due to the amazing guidance of my supervisor and her colleagues, I am now a lot more comfortable with this type of work.

During this project, I have gained more experience with the basics of working in the lab, as well as having been introduced to some new techniques and equipment. I would recommend a similar project to others who are interested in working in the lab.

6 Future work

Due to the limited amount of time for the project, we did not get to complete all the desired tests. Since there is a lot more one can do with this project, I will be able to expand upon this work in my Specialization project next semester. Optimizing the process, performing other kinds of tests and trying different combinations of enzymes and carbohydrates is something that can be done in future work.

References

[1] Oliver, C. M., Melton, L. D., Stanley, R. A. (2006). Creating proteins with novel functionality via the Maillard reaction: A review. Critical Reviews in Food Science and Nutrition, 46, 337–350.

[2] Kato, A.; Murata, K.; Kobayashi, K. Preparation and characterization of ovalbumin-dextran conjugate having excellent emulsifying properties. J. Agric. Food Chem. 1988, 36, 421- 425.