MIM JOURNAL 2019 illuminate your work
Made in Millersville Journal | www.mimjournal.com

Development of an Aptasensor

Simona Gavrilovici

Abstract

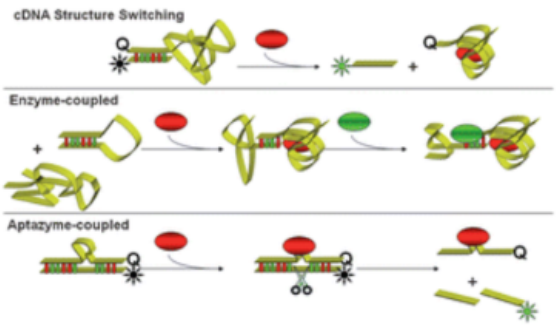
The objective of this study is to develop an aptasensor that can quantitatively detect the specific target myoglobin molecule. Once this is achieved, the aptasensor could be further developed in biomedical studies to detect more critical targets like mutated cells that could cause cancerous or even dangerous bacterial infections. Fluorescence aptasensors are highly sensitive and selective. The targeted chemical is introduced to the fluorophore-labeled aptamer. The fluorophore-labeled aptamer fluorescence signal changes in response to the aptamer binding to its target. This allows for the quantitative determination of the target molecule. Raman spectroscopy is a second method that can also be used to detect the binding of an aptamer to its target molecule. The Raman signal can be enhanced to increase sensitivity using surface enhanced Raman spectroscopy (SERS).

Background

Aptamers in Biochemical and Pharmaceutical Research

Aptamers are artificial single-stranded DNA or RNA sequences that fold into secondary and tertiary structures that bind to certain targets with extreme specificity. The ability to have a high selective affinity gives it a resemblance to chemical antibodies. The body creates antibodies to fight off foreign objects, like the cold bacteria or the flu. In laboratories, antibodies could be synthesized through chemical processes. However, aptamers have several unique characteristics that make them more effective than antibodies. For instance, the use of aptamers overcomes the limitation of having to use **cell lines**, as is necessary for antibodies. The use of aptamers instead of antibodies in biochemistry and pharmaceutical research could further enhance studies on drug development (Iliuk, 2011).

Aptamers are generally produced using the conventional SELEX procedure. First, magnetic beads are used for target immobilization to facilitate highly efficient separation of the binding DNA aptamers from the unbound DNA. Then, **flow cytometry** and **fluorescein labeling** are used to monitor the enrichment (Wang, 2009). The procedure has repeated rounds of **in vitro selection**. A random library containing approximately 1000 single-stranded DNA or RNA sequences is used for DNA aptamer separation. The library is incubated with the chemical target of interest to start the first cycle of selection. This is followed by **iterative cycles of absorption**, recovery of bound DNA or RNA, and **amplification** (Iliuk, 2011).



Scheme 1. The process of synthesizing an aptamer via the SELEX approach with repeated rounds of in vitro selection (Sefah, 2009).

The bounded DNA or RNA is separated from the unbounded DNA or RNA through a separation technique, like **affinity chromatography**, to ensure purity and selectivity (Iliuk, 2011).

Fluorescent Probe

To detect the aptamer and subsequent binding to a target molecule the use of **reagents** and **spectroscopic probes** are necessary (Iliuk, 2011). **Fluorescence spectroscopic** methods have high sensitivity and a millisecond time scale of their application. Since the intrinsic fluorescence spectroscopy of normal RNA bases are very weak, there have been recent studies of nucleic acids that relied on the introduction of **extrinsic fluorophores** used in probes. Since most fluorophore tend to be bulky molecules with low water solubility, quantitative labeling efficiency is required for many fluorescence applications (Qin, 1999). Aptamers are **oligonucleic acid molecules** that can be readily modified with fluorescence tags (Iliuk, 2011).

Raman Spectroscopy

Raman Spectroscopy can also be used for the development of an aptasensor. Prominent features appear in Raman spectra that are unique to a specific chemical, which aids in the identification and characterization of the chemical (Dresselhaus, 2008). Furthermore, Raman spectra can show extreme sensitivity when using **surfaced enhanced Raman spectroscopy** (SERS) (Sivanesan). Important parameters associated with SERS is that there is **homogeneity** of the **substrate** used and selectivity towards its target molecule (Sivanesan, 2015). The surface enhanced Raman scattering properties of nanoparticles have promoted rapid development of Raman spectroscopy. The two frequently used active substrates for SERS are metal colloids and electrodes with rough surfaces. SERS will aid in the development of a high sensitivity aptasensor (Ma, 2018).

Experimental Results

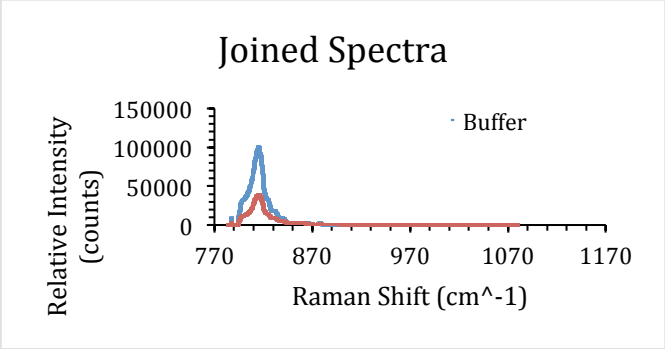


Figure 1. The Raman spectrum of the **myoglobin** added to the **buffer solution** is compared to the Raman spectrum of the buffer solution.

Conclusion

The procedure of the development of an aptasensor is still undergoing experimental trials. The buffer that will be used for the apatasensor and the target myoglobin molecule was analyzed with Raman spectroscopy. In **Figure 1** there is little to no difference between the Raman shift of the myoglobin and of the buffer. The buffer solution has Raman signals that are high in relative intensity, therefore there is no way

to distinguish the myoglobin signals in the Raman shift region of 790 cm^{-1} to 840 cm^{-1} from the buffer signals. Trials are conducted with changes in the Raman parameters in an effort to separate the buffer signals from the Myoglobin signals.

Acknowledgments

I would like to thank the funding and support for this project from Niemeyer-Hodgson Student Grant, Student Grant for Research and Creative Activity Committee, Millersville University ACS Student Chapter, project mentor Dr. Lyman H. Rickard, and the Department of Chemistry.

Glossary

Affinity chromatography is a method for separating biochemical mixtures based on specific interactions between biochemical molecules (p.3).

Amplification is the process of adding an enhancement to a value or object (p.3).

Buffer solution is a liquid that has a pH balancing quality. This means that it prevents the entire solution from becoming too acidic or too basic (p.4).

Cell lines are clone cells of a tissue sample that had been cultured in a laboratory (p.2).

Enrichment is the amount of a particular molecule that is occupying a particular sample space or cell (p.3).

Extrinsic fluorephore is a fluorescent dye that could be made of small molecules and is added to biochemical substances for fluorescence spectroscopy (p.3).

Flow cytometry is a method used to detect and measure both physical and chemical properties of a population of cells (p.3).

Fluorescence spectroscopy is an analytical technique used to measure the absorbance of electromagnetic radiation in a sample solution containing fluorescent agents and the sample solution (p.3).

Fluorescein labeling is the addition of a fluorescent agent to the sample solution. This agent will illuminate the solution when it absorbs a specific wavelength from the electromagnetic spectrum (p.3).

Homogeneity is achieved when the solution has the same composition everywhere within its sample cell (p.4).

In vitro selection is the study of biological substances outside their normal context (p.3).

Iterative cycles of absorption are the repetitive measurements of the absorbance of a sample solution to increase precision of the experimental results (p.3).

Myoglobin is red blood cells that do not have an oxygen molecule bound to it. They carry oxygen to the tissues (p.4).

Oligonucleic acid molecules are short DNA or RNA molecules (p.4).

Raman Spectroscopy is an analytical method used to measure the vibrational intensity of a molecule as detected by the excitation of the electrons with an infrared radiation source (p.2).

Reagents are the chemicals used with the starting material to create a reaction (p.3).

Spectroscopic probes are used to improve sensitivity in an analysis of a chemical substance (p.3).

Substrate is the material that is under investigation. It is usually synthesized into a product using reagents in a chemical reaction (p.4).

Surface enhanced Raman spectroscopy is Raman spectroscopy that uses metallic nanoparticles to improve the signals detected by the ramanspectrometer that is emitted from the irradiation of the solution being studied (p.4).

References

Agee, J. (2005). Literacy, aliteracy, and lifelong learning. *New Library World, 106*(5/6), 244 252. doi:10.1108/03074800510595850

Chong, S. L. (2016). Re-thinking aliteracy: When undergraduates surrender their reading choices. *Literacy*, *50*(1), 14-22. doi:10.1111/lit.12063

Little, C. A., McCoach, D. B., & Reis, S. M. (2014). Effects of differentiated reading instruction on student achievement in middle school. Journal Of Advanced Academics, 25(4), 384-402. doi:10.1177/1932202X14549250

Recommended Citation
Gavrilovici, S. (2019). Development of an aptasensor. *Made in Millersville Journal*, 2019. Retrieved from https://www.mimjournal.com