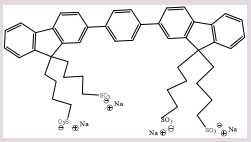
Negatively Charged Conjugated Oligomer as Part of a Pathogen Biosensor

Danielle F. Okerblom, A. Gonzalez, G.C. Bazan*
Department of Chemistry and Biochemistry, UCSB
email: seastar15142@yahoo.com

Goal/ Hypothesis:

We created a biosensor composed of an conjugated, anionic water soluble oligomer and cationic peptide tagged with a FAM (flourescene) with the intention of using it to detect pathogens. The mechanism was expected that the electrostatic interactions of the cation and anionic molecules will interact to form ball like aggregates. The oligomer has a -4 charge and the peptide human platlet factor 4 (HPF) has a +7 charge. In these aggregates energy transfer occurs from the chromophore of the negative oligomer to the fluorescene on the postive peptide because when the proximate is less than 10nm. The stable, FRET spectral signal can be measure with a fluorimeter. Using these results we will be able to determine the presents of bacteria.



Oligomer: $FPFC_4S0_3^- Na^+$

Methods & Material:

All data was recorded with a Shimadzu UV-2401 PC diode array spectrometer. In each experiment the oligomer was excited at 350 nm and the emission was collected from 362-650 nm. Samples were prepared to the following peptide oligomer ratios:

HPF to $FPFC_4S0_3$ ratios	Pep/Olig 1.0:1.10	Pep/Olig 1.0:1.25	Pep/Olig 1.0:1.50	Pep/Olig 1.0:1.75
Conc. with respect to charge	9.093/8= 1.136	9.625/8 = 1.203	11.887/8= 1.486	14.004/8= 1.751
Conc. with respect to molarity	1.299/2 = 0.650	1.375/2= 0.6875	1.698/2= 0.849	2.000/2= 1.00

Data/Results:

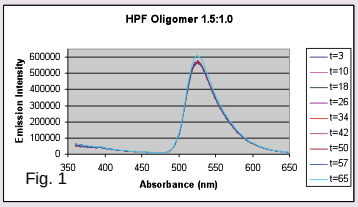


Fig. 1

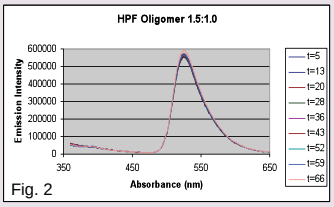


Fig. 2

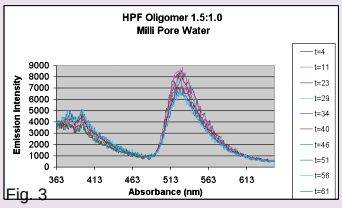


Fig. 3

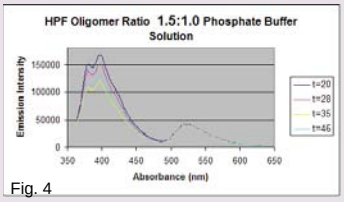


Fig. 4

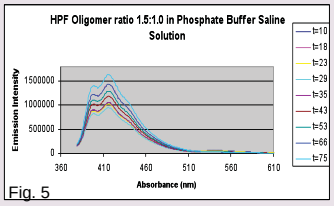


Fig. 5

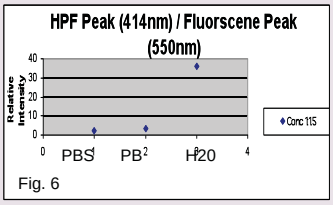


Fig. 6

Figures 1, 2 and 3 illustrate the reliability of the fluorescence peak due energy transfer when the oligomer is excited. Figured 3, 4 and 5 illustrate the same ratio of peptide to oligomer in different solvents. Figure 6 shows the relative intensity of fluorescene emission peak divided by the oligomer emission peak shown in the previous graphs for each solvent.

Summary/Future Work:

The data shows that energy transfer does not occur sufficiently in either the phosphate buffer or the phosphate buffer saline. Millipore water will be used as the solvent for future experiments. Background measurements so far indicate that to determine the presents of bacteria we will compare the signal of solution before and after addition of a bacteria to solution. The signal will be considered stable after 15 minutes of agitating. Our further experiments include:

1. Measuring the interaction of the oligomer with other peptide
2. Addition of pathogens to the biosensor in solution
3. Measurement of a non-tagged peptide to determine if the peptide itself quenches the oliogmer energy transfer
4. Further research of ideal concentrations once pathogen is added.

Conclusion:

What we found is that the signal is most stable in Millipore water. Prior knowledge and finding so far indicate that the pattern is reliable and when we are ready to introduce a pathogen. It appears that 1.0 : 1.50 and 1.0 : 1.25 ratios are sufficient concentrations for aggregations to occur. In the future we expect that in the change in signal will lead to a differentiation of between different pathogens.

Acknowledgements:

INSET

The Okerblom Family for their support

Research Funding Provided by NSF

Allan Hancock College

Kathy Jimison MS, Cuesta College

Thank you all without your support I would not be here.