Determination of Capsaicin Concentration in Aqueous Solution by use of Luminescent Lanthanide Chelates.

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Abstract

Capsaicin is the molecule that creates the burning sensation when consuming peppers. It is of particular use to the medical field because of its pain-relieving qualities. A series of diethylenetriamine (DTPA) based chelates where made to measure the concentration of capsaincinoids in solution. Terbium is a lanthanide that was used with these metal chelates to create a luminescent response. Luminescent titrations of both terbium as well as a simplified DTPA molecule were taken and enhancement was recorded in both trials. This simplified DTPA was modified to better bind with capsaicin by modification of functional groups. Unfortunately the more complex DTPA chelates were not water soluble therefore luminescent data for these compounds could not be obtained. Although the products were unable to perform the desired task this project can be described as a success. The synthesis of these molecules went as planned as can be seen by the 1HNMRs and since these molecules had never been made before this research gives conclusive results.

Introduction

Capsaicin is a naturally occurring vanilloid found in peppers. Capsaicin creates a burning sensation when consumed by activating the TRPV1 protein. This protein can also be activated by high temperatures thus describing the burning sensation of capsaicin. Capsaicin has three distinct functional groups as can be seen in (fig. 1). The benzene ring, polar group, and hydrophobic chain make capsaicin look similar to soap in structure. The length of the hydrophobic chain greatly alters the pungency of the molecule. The longer the
chain of the cap the more potent the burning sensation is. Cap 1 and Cap 5 (fig 2) are synthetic molecules that resemble the structure and function of naturally occurring capsaicin. The TRPV1 gene is located throughout the body making capsaicin of interest to the medical field due to its pain relieving potential (1). Currently HPLC is the primary detection method for capsaicin. The ultimate goal of this research would be the development of an alternate quantitative detection method using luminescent lanthanide chelates in solution.

**Fig 1. Capsaicin structure**

Chelates are useful in the detection of capsaicin in solution. Ligands are any atom or molecule coordinated to a metal ion. A single molecule with many coordination sites able to bind to the metal is referred to as a chelate. Since water has only one electron donating atom it is not a chelate. Lanthanides are a family of molecules located in the 6th row of the periodic table. Lanthanides are useful because their unusual spectroscopic properties; they display absorption and emission bands that correspond to laporte-forbidden transitions which results in distinct peaks in luminescence excitation and emission testing. In solution these molecules exist exclusively in the 3+ state because of hydration enthalpy. These lanthanide ions prefer oxygen donors and only form stable aqueous solutions with chelates because they have very low molar absorptivities which make the direct excitation of the metal difficult. (2). Since the 4F orbitals are not affected by the ligand these excitation and emission bands are precise and distinctive of the individual metal regardless of chelate. Terbium was the chosen lanthanide for this research but others such as europium could also be used because the properties are so similar.
Chelates are used to increase the luminescence of the lanthanides by taking advantage of the antenna effect (fig. 3). Capsaicin binds to the chelate portion of the chelate-lanthanide complex which transfers the energy to the lanthanide. This makes the fluorescence measurable by extending the lifetime. Water quenches this effect because de-excitation of lanthanides may occur through non-emissive pathways like OH bonds while other molecules may enhance the luminescence (3). Using the above information, luminescent responses can be used to determine concentration or binding strength using a fluorimeter.

![Fluorescent Signal](image)

**Fig 3. Antenna effect**

In order to accurately measure capsaicin concentration any activity from other molecules in solution must be somehow averted. Synthesizing a chelate that strongly binds to capsaicin by targeting the functional groups of the desired molecule is one such way to ignore effects of other molecules in solution. This research focuses on the multistep synthesis of chelates that strongly bind to terbium while also interacting with the functional groups of capsaicin. Diethylene triamine pentaacetic acid, or DTPA, is a readily available metal chelate which acts as a perfect starting point. Two of the five carboxylic acids are converted to amides by a nucleophilic acyl substitution to give DTPA-BMA (fig. 4). The red molecules represent those binding to the terbium while the blue are the R groups that will
be changed to better bind to capsaicin. Also below is an example of a final target product, (fig 5) notice how the chelate arms resemble the structure of capsaicin, a benzene ring followed by a hydrogen bond donor/acceptor pair and a hydrophobic region. Following the age old rule of like dissolves like this makes this structure an excellent candidate to strongly bind to capsaicin and create a signal from the lanthanide. A fluorescent titration of the chelate/Terbium complex with capsaicin is run to verify results. Excitation spectrums are run at an emission wavelength of 545 nm and Emission spectrums are run at an excitation wavelength of 295 nm (4). By using known calibration curves the capsaicin concentration can easily be determined in aqueous solution using just a drop of product. All products will be analyzed using 1H-NMR, specifically the Varian EM360L NMR spectrometer. All Fluorescent data will be gathered using a Varian Cary Fluorescence Spectrophotometer.

**Procedure**

**DTPA-BA (I-1)** was synthesized by combining 18.82g (47.9mmol) DTPA with 22 mL acetic anhydride and 35 mL pyridine in a 250 mL round bottom flask fitted with a water condenser. The solution was heated overnight at about 60 C. After 16 hours the solution was black with a tan precipitate. The product was vacuum filtered washed with acetic anhydride then ethyl ether to yield a tan powder. 15.8g 92.4% yield.

**4-nitrobenzylphthalimide (I-3)** was synthesized by combining 50g (231mmol) 4-nitrobenzyl bromide and 47.1g (255mmol) K phthalimide in 600 mL roundbottom flask then adding 150 mL DMF. The solution was transferred to a 1 L erlenmeyer flask and 400
mL water was added to remove DMF and salt. This solution was vacuum filtered to leave product. 62.0g 95% yield.

**P-nitrobenzylamine (I-5)** was synthesized by combining 62g (219mmol) I-3 with methanol in a 1000 mL beaker with slow heat and stir. 35 mL (721mmol) hydrazine-hydrate was added to form a large white precipitate. 350 mL CH$_2$Cl$_2$ was added to further precipitate then the solution was vacuum filtered keeping the liquid as our product is dissolved. It was dried using MgSO$_4$ then mixed with 700 mL CH$_2$Cl$_2$ and gravity filtered. This liquid was placed in a rotavapor until dry. 28.24g 84% yield.

**DTPA-BMA (I-9)** was synthesized by combining 2.144g (6mmol) I-1 and 1729 uL (30mmol) 40% w/w CH$_3$NH$_2$ in H$_2$O. 14 mL DMF was added and the solution was allowed to stir overnight. The solution was then placed in the rotavapor until dry. Yield not recorded.

**DTPA-Nitrobenzyl R group (I-13)** was synthesized by dissolving 5.2g (34mmol) P-nitrobenzylamine in 25 mL DMF in a 250 mL round bottom flask. Separately, 4.5g (12.6mmol) DTPA-BA (I-1) was dissolved in 25 mL DMF before adding 8 mL triethyl amine. These solutions were combined and stirred overnight. The solution was put on the rotavapor until dry and then this solid was acid crystallized to a pH of 4. The liquid was poured off the solid was dissolved in methanol and put on the rotavapor twice to purify. 5.65g 67.8% yield.

**DTPA- hydrogenation of I-13 (I-15)** was synthesized by combining 9.26g (14.0mmol) I-13 and 3.0g Pd/C in a 500 mL round bottom flask fitted to stir. helium balloons were used to remove air from round bottom and 240 mL MeOH was syringed in under helium pressure and stirred overnight. This product was put on the rotavapor to dry. Yield was not recorded.
**DTPA- bisisothiocyanate (I-17)**

**DTPA-butyl bisthioure (I-27)** was synthesized by dissolving .032g (.44mmol) butylamine in 2 mL MeOH in a vial. In a round bottom flask combine .144g (.2mmol) I-17 and .041g (.45mmol) Na$_2$CO$_3$ then add 4 mL water and 2 mL MeOH. Pipett vial solution into round bottom while stirring. The solution was boiled to remove methanol then put on the rotavapor and high vac before being acid precipitated out of water. This was vacuum filtered and the paper was put on the high vac then left to dry in desiccator overnight. 59mg 36% yield.

**DTPA-octyl bisthioure (I-29)** was synthesized by dissolving .057g (.44mmol) octylamine in 2 mL MeOH in a vial. In a round bottom flask combine .144g (.2mmol) I-17 and .041g (.45mmol) Na$_2$CO$_3$ then add 4 mL water and 2 mL MeOH. Pipett vial solution into round bottom while stirring. The solution was boiled to remove methanol then put on the rotavapor and high vac before being acid precipitated out of water. This was vacuum filtered and the paper was put on the high vac then left to dry in desiccator overnight. 49mg 28% yield.

**DTPA-butyl bisthioure/Tb$^{3+}$ complex (I-31)** was synthesized by dissolving 59mg (.068mmol) I-27 in 2 mL water in a 25 mL round bottom flask. Then 284 uL of a solution with 16.2 mg NaOH and water was added before adding 2 mL MeOH. Separately, 26mg (.068mmol) TbCl$_3$$^*$$^6$H$_2$O was dissolved in .5 mL water before adding 1 mL MeOH. These solutions were combined and stirred overnight. 15mg 21% yield.

**DTPA-octyl bisthioure/Tb$^{3+}$ complex (I-33)** was synthesized by dissolving 40.7mg (.044mmol) I-29 in 2 mL water in a 25 mL round bottom flask. then 186 uL of a solution with 7.95 mg NaOH and water was added before adding 1 mL MeOH. Separately, 16.8mg
(.044mmol) TbCl$_2$·6H$_2$O was dissolved in .5 mL water before adding 1 mL MeOH. These solutions were combined and stirred overnight. 29mg 58% yield.

DTPA Scheme

Results $^1$HNMR and luminescent titration
Fig 8.

Fig 9.
Fig 10.

Fig 11.
Fig 12.
Fig 13.

JWF-I-19 in D2O w Na2CO3 (512 scans)

DTPA -butylbisthiourea

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8 7 6 5 4 3 2 1 0 1 2 3 4 5 6 7 8

A B C D E

8.09 5.29 5.01 3.83 2.82

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Excitation Spectrum of Cap5 in TbCl₃ (λₑₑ = 545 nm)

Fig 14.
**Fig 15.**

**Discussion**

Fig 6 clearly shows that DTPA-BA was produced as desired. Notice the 8 hydrogens described by peak A; this is a distinct singlet that would not appear in the $^1$HNMR of regular DTPA. Figure 7 is not a follow up to fig 6, but rather a separate reaction. The region at 8 ppm is indicative of multiple benzene ring carbons being in similar environments. Fig 8 shows that this region is much more distinct which is due to the fact that the carbons on the benzene ring have much more distinct environments so their signals do not overlap. Fig 9 shows the NMR results for DTPA-BMA. Although there are many solvent peaks this data is still valuable by comparing to the previous NMR of DTPA-BA. Compared to fig 6, fig 9 is missing that distinct singlet peak at 3.9 PPM which indicates that this reaction ran to completion. Fig 10 shows the doublets around 8 PPM which signifies that the nitrobenzyl group was indeed added to the chelate. The main
difference between fig 10 and 11 is that fig 11 has the doublets at around 7 PPM instead of 8 PPM. This change is indicative of transforming a strongly electron-withdrawing group into a strong electron-donating group which is exactly what was done in this hydrogenation. Fig 12 shows the same basic structure that fig 11 showed which is to be expected as only one group was changed and it was not attached to any hydrogen containing carbons. Fig 13 is difficult to see but is useful to compare to the desired molecule to make sure there are no glaring differences. Fig 14 shows a control luminescent titration of TbCl$_3$ with cap 5 and no chelate. While luminescent enhancement was observed it was nowhere near as much as with a metal chelate present. Unfortunately the octyl and butyl complexes did not dissolve so luminescent data was not obtained for the compounds. However, the luminescent potential of these products can be seen by looking at the results of a simpler version, fig 15, which did dissolve. At the peak concentration of capsaicin (1.6 eq) the DTPA-BMA complex showed a 7 fold increase in emission at the 545 nm wavelength which has been attributed to terbium in past experiments. The fact that the max was observed at 1.6 equivalence leads to a belief that this is a 1 to 1 binding ratio which has been noted in previous experiments (5).

**Conclusion**

To sum up the experiment, DTPA based chelates have potential as luminescent signalers but much work is required in the future before a reliable technique could be determined using the molecules designed in this procedure. All desired products were synthesized and verified using HNMR but the key data of interest was unobtainable due to solubility issues. Luminescent testing was done on terbium as a control and also on a simplified version of our target chelates to show the potential for these molecules in the future. The octyl and butyl derivaties need to
be converted to more water soluble forms or another solvent other than methanol and water needs to be tested so that research into these DTPA complexes can continue. In future work extra care needs to be paid attention to measurements and procedures as errors multiply in a multi-step procedure.

Cited


