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A NEW GENERATION OF FLUORESCENT CHEMOSENSORS DEMONSTRATE IMPROVED ANALYTE DETECTION SENSITIVITY AND PHOTOBLEACHING RESISTANCE

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Abstract: Molecular chemosensors have found increased utility in the development of precise and sensitive detection devices. However, chemosensors that report binding via fluorescence through UV excitation are susceptible to destruction via photodegradation of the fluorophore. In the following report, the dansyl fluorophore in a previously reported chemosensor for peptides is replaced with an acridone derivative that is highly resistant to photobleaching. Its spectral properties are closely matched to those of the original dansyl fluorophore, and although quite structurally dissimilar, the new more photostable acridone chemosensor analogue exhibits only minor differences in binding/detection characteristics.

Introduction

Sensitive qualitative assessment of binding strengths are necessary for useful comparisons of binding ligands. Chemosensors,[1,2] molecular devices that sense and report its binding avidity toward molecular targets, may be used in such a qualitative manner. The chemosensor device that we describe here incorporates a binding pocket comprised of two macrocyclic arms attached to a pyrrolidine core which change their configuration upon analyte binding (Figure 1). This binding interaction induces a change in distance between a fluorophore attached to one of the macrocyclic arms and a quenching moiety attached to the core. These are engaged in a Forster type interaction (fluorescence resonance energy transfer, FRET) such that the level of fluorescence that escapes quenching is proportional to the binding strength (Figure 2). However, in these devices repeatable consistent fluorescence output is a necessity for obtaining dependably sensitive detection of binding. Unfortunately, decomposition of fluorophore by photobleaching is a significant source of detection error, and limits the useful life of a chemosensor.

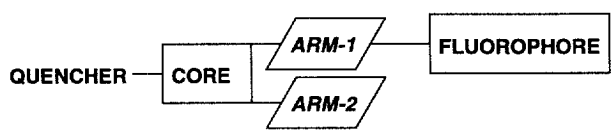

Figure 1. Modular receptor construction

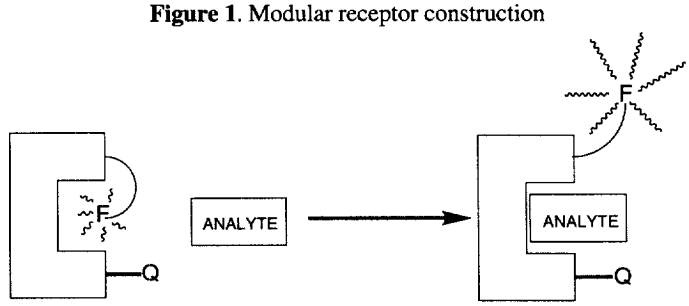

Figure 2. Fluorescence increase upon binding

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Chemosensors for peptides developed previously by this group utilized a dansyl fluorophore. Although dansyl moieties are known to be susceptible to photobleaching, this work was not appreciably affected due to short UV exposures for fluorescence measurement. It is likely that long UV exposure conditions or repeated use of a chemosensor may be required in future projects which would cause inaccurate fluorescence measurement. Since the magnitude of error is dependent upon the total irradiation time, there is clearly a need for more photostable fluorophores. A much more photostable fluorophore (and capable of similar emissions characteristics) such as acridone[3] would be more suitable for repeated accurate measurement with extensive irradiation.

Background

The original series of these chemosensors,[4] utilized a dabcyl (4-((4-(dimethylamino)phenyl)azo)-benzamide) quencher, *Q*, and a dansyl sulphonamide fluorophore, *F*, attached to a macrocyclic structure (Figure 3). This structure is comprised of a pyrrolidine core attached to two macrocyclic isophthalamide arms, which are configurationally restricted to allow purposeful binding. Through previous peptide library scanning studies the peptide, Ac-*D*-Pro-*L*-Val *D*-Gln(Trityl)-O_iPr, was found to bind well to this macrocycle.[5]

It is important to note that fluorescence of the unbound chemosensor shows 99.6% quenching in comparison to the fluorescence output of the neat dansyl sulphonamide fluorophore.[4] However, upon binding its complementary ligand the fluorescence is quenched by only 97.2%. Although the change in quenching is not expansive, there is still a fivefold increase in fluorescence intensity upon binding saturation as seen in Figure 5.

Although these solution phase results appear satisfactory, difficulties arise upon long term UV irradiation of this receptor system on solid support. In practice, quencher-fluorophore equipped receptors may be used to scan solid-phase libraries whereby it binds to particular library beads carrying a complementary substrate. The prolonged exposure required to identify and isolate these solid support beads tends to cause photobleaching of the dansyl moiety, thus decreasing fluorescence. Previous experience with dansyl fluorescent receptors has shown photobleaching to be so detrimental that it was only possible to identify and isolate a fraction of the receptor-selected beads in a given optical field.

As an initial test, the photobleaching properties of acridone and dansyl sulfonamides were demonstrated by prolonged exposure of these compounds to UV radiation. Since the fluorophores are eventually to be used on solid support, they were subjected to UV bleaching in solid form absorbed onto filter paper. After 12 h exposure to 365 nm from a 160 mA mineral lamp at 0.5 cm distance, the acridone retained 95% of its fluorescence while the dansyl sulfonamide retained only 43%.

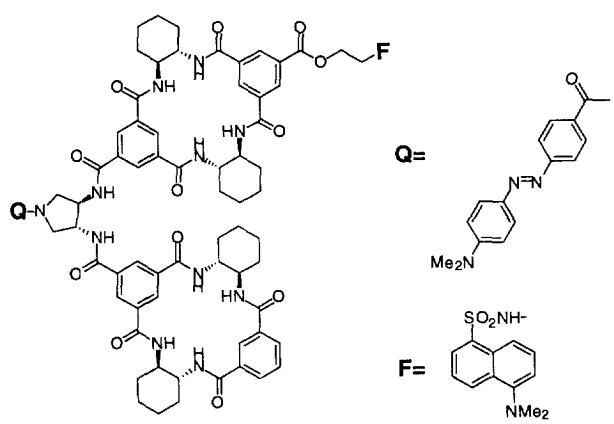

Figure 3. Receptor structure

Results

The work described here concerns the direct replacement of the dansyl fluorophore moiety 2 with an acridone derivative 6.

The dansyl sulfonamide 1 was obtained through the commercially available dansyl chloride. As shown in Figure 4, the dansyl chloride reacts with ethanolamine to produce the sulfonamide alcohol which is then used to esterify[4,5] Arm-1 (Figure 1). The acridone derivative was procured through commercially available acridone 3. This was first methylated via NaH, and MeI to yield the *N*-methyl derivative 4. The sulfonyl chloride derivative 5 was obtained by dissolving 4 in excess chlorosulfonic acid for 24 h with its subsequent slow addition to chilled aqueous $NaHCO_3$. The solvent was evaporated, and the residue extracted and recrystallized from hot acetone to yield the product. The sulfonamide 6 was procured in the same manner as for the dansyl derivative.

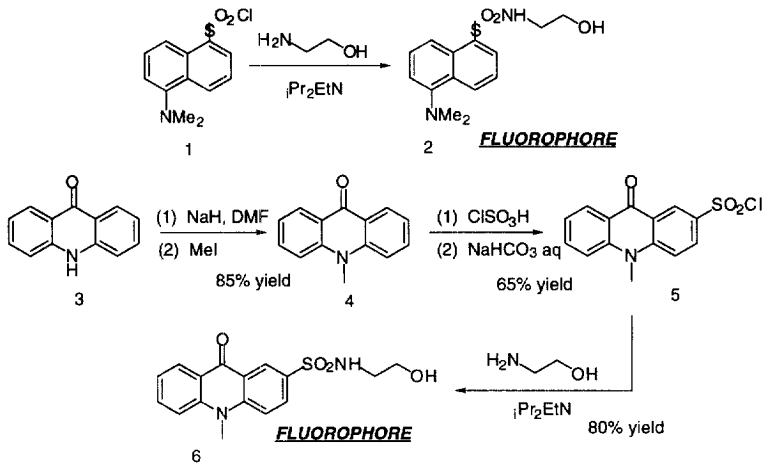

Figure 4

Fortunately, the profile of the emission spectrum of acridone attached to the receptor and its overlap with the dabcyl absorption spectrum is similar to that of the dansyl derivative as indicated by the similar values of their overlap integral and Forster distance (Table 1). Indeed, as demonstrated by peptide binding-saturation experiments (Figure 5) the acridone-receptor derivative saturates in the same fluorescence profile as the dansyl-receptor.

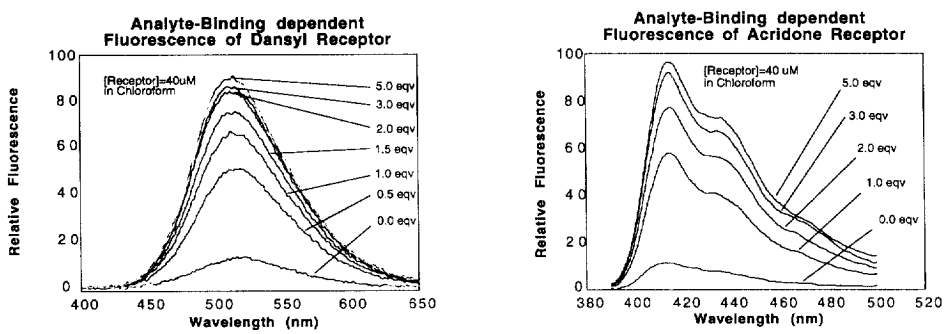

Figure 5. Receptor binding-saturation experiments

Forster theory accurately describes the ideal emission-quenching characteristics for the two receptor systems. From the quenching and fluorescence spectra one is able to determine the Forster distance,[6,7] the characterstic distance between quencher and fluorophore that allows half of the fluorophore emission to be quenched.

Table 1. Forster calculation results

F-Q pair of receptor	Dansyl-Dabcyl	Acridone-Dabcyl
Förster Distance, Å	33.1 Å	36.3 Å
J ($10^{-14}cm^3M^{-1}$)	8.22	5.14
F-Q distance for bound receptor, Å	18.8 Å	18.8 Å
F→Q energy transfer efficiency for bound receptor	0.972	0.982
F-Q distance for empty receptor, Å	13.6 Å	12.8 Å
F→Q energy transfer efficiency for empty receptor	0.996	0.998

From the determined Forster distance for a particular fluorophore-quencher pair and the fluorescence measurements of receptor binding experiments one is able to obtain the average fluorophore-quencher distances of a particular receptor system in both the bound and unbound states. Although the quencher-fluorophore distances for the bound and unbound states as calculated above are significantly less than the calculated Forster distances, the small quenching change upon binding still allows sevenfold increase of fluorescence intensity for the acridone type receptor and a fivefold increase of fluorescence intensity for the dansyl type receptor. It is also worth noting that the changes in fluorophore quenching between bound and unbound states for both receptors is relatively small with respect to their corresponding changes in fluorophore quenching distances (Table 1). Since the maximal changes in fluorescence quenching occur when changes in these distances transpire near the Forster distance, greater fluorescence changes would occur upon binding if the Forster distance for the system were located near the calculated fluorophore-quencher distances for the bound and unbound states of these receptors.

Conclusion

Replacement of the dansyl moiety of the previously described receptor with an acridone derivative creates a receptor with more resistance to fluorophore photobleaching without significantly changing its binding-saturation characteristics. Since the binding constant K_a's of the peptide ligand to each of the receptor systems and their binding saturation profiles are similar, it would be likely that the configurational changes of each receptor would be similar upon binding. For this reason the fluorophore-quencher distances, in the unbound and bound saturation forms, ought to be similar between the dansyl and acridone type receptors. Indeed, the fluorophore-quencher distances are consistent between receptors (Table 1). However, more significantly, the acridone receptor exhibits increased fluorescence output upon binding saturation and higher bleach resistance, without changing the Forster characteristics.

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