Fluorescence Anisotropy as a Measure of Chiral Recognition

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The study of chiral interactions is one of the most challenging and formidable of scientific endeavors. From the time that Louis Pasteur first proposed what was to become the foundation of stereochemistry, scientists have sought a complete understanding of chiral interactions. The results of such investigations are far-reaching, ranging from the development of methods for physical separation of optical isomers to the development of new biologically active pharmaceuticals. Despite the considerable effort and accomplishments that have been made in this area,[1] a precise description of the phenomenon of chiral recognition has yet to be realized. Advances in the understanding of chiral recognition are of immediate interest to those working in the areas of drug design and synthesis, separation chemistry, and chemical sensor development. Therefore, a need exists for the development of novel analytical methods to examine chiral interactions. Such advances bear significant relevance to a broad segment of the scientific community.

There are relatively few examples of enantioselective photophysical behavior in the condensed phase.[2] The majority of these examples are based on enantioselective quenching[3] and excimer formation,[4] or spectral shifts[5] upon host/guest complexation. While these advances are significant, a disadvantage of these approaches is that they are dependent on specific photophysical properties of the analyte, which are typically not broadly applicable.

Fluorescence anisotropy is a spectroscopic technique that has been widely used to study molecular interactions, particularly in biological systems.[6] To our knowledge, however, there are few reports on the use of fluorescence anisotropy to specifically study enantioselective interactions and chiral recognition. Recently, Al Rabaa et al.[7] reported the spectroscopic characterization of a chiral anthryl probe and its enantioselective interactions with DNA. Herein, we detail an approach to examine and quantify the phenomenon of chiral recognition using fluorescence anisotropy.

Fluorescence anisotropy is a polarization-based technique that is, among other factors, a measure of the rotational motion of a fluorescent molecule. The fluorescence anisotropy is affected by intrinsic and extrinsic parameters, the latter of which is of primary interest to this study. Specifically, rotational diffusion of the fluorophore is typically the dominant depolarization mechanism. In this manner, the fluorescence anisotropy is sensitive to the size of the rotating body. When a fluorescent molecule is free in solution, it will rotate at a rate commensurate with its size. If, however, it forms a complex with another species, its rotational rate will be coupled to that of the entire complex, the degree to which depends on the strength of the binding interaction. This phenomenon has led to the widespread use of fluorescence anisotropy to examine binding interactions, for example, in immunoassays.[8]

Consider the case of a chiral fluorophore in the presence of a discriminating chiral selector. One enantiomer will bind more strongly, resulting in a coupling of the rotational motion of both species. The larger volume of the complex will presumably result in a slower rotational correlation time, hence, a higher anisotropy value. The interaction of the fluorophore with the chiral selector, especially in the case of chiral surfactants, will undoubtedly involve nonstereoselective interactions, as well as the expression of chiral selectivity. These nonstereoselective interactions will, by definition, be identical for both enantiomers. Therefore, any difference observed in the fluorescence anisotropy should be due to the chiral interaction. In fact, the magnitude of the difference should be a good measure of chiral recognition. This preliminary study investigates the validity of the preceding hypothesis by examining the enantioselective interactions of four chiral analytes with a dipeptide-based polymeric micelle as a chiral selector.

In this study, paired solutions were prepared to measure separately the steady-state anisotropy of the enantiomers in the presence of the chiral selector. The analyte concentration was 1×10^{-4} M. Both solutions were prepared from the same stock solution of chiral selector (1.3% (w/w)) with the exception that one solution was prepared with the R enantiomer of the analyte, while the other was prepared with the S enantiomer.

In the case of 1,1′-bi-2-naphthyl-2,2′-diyl hydrogen phosphate (BNP) in the presence of a chiral selector,[9] the R enantiomer had an anisotropy value of 0.1132 ± 0.0003, and the S enantiomer had an anisotropy of 0.0991 ± 0.0004. While the magnitude of the anisotropy difference is expectedly small, it is clear that the difference is significant. To confirm enantioselectivity as the source of the anisotropy difference, the two enantiomers were measured in the presence of an achiral polymeric micelle, polysodium undecanoyl glycinate. In this system, the anisotropy values of both enantiomers were identical.

In light of the aforementioned findings, fluorescence anisotropy appears to be a good measure of chiral recognition within these systems. It is difficult, however, to compare the anisotropy values from one system to another because the enantioselective interaction is only one of several factors that affect the observed anisotropy. These factors include the intrinsic anisotropy, the fluorescence lifetime, the partitioning of the fluorophore in the micellar system (i.e., solubility), and the size of the host/guest complex. Fortunately, these nonenantioselective factors are identical when comparing two enantiomers of a given analyte. However, these factors are likely to differ with various analytes and chiral selectors and under various experimental conditions.

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(9) (L,L) poly sodium undecanoyl leucyl-leucinate (poly-SULL) was used as the chiral selector. Poly-SULL is one of a class of polymerized dipeptide surfactants being developed that have been demonstrated as good chiral selectors in MCE.

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Table 1. β-Values for Analytes in the Presence of poly-SULL

analyte	BOH	BNA	BNP	Troger's base
β-value	4.5	7.1	17.8	24.2

To arrive at a term that can be used to compare chiral selectivity among various host/guest systems, we have chosen to represent the chiral selectivity as the term, β. The term β relates the difference in observed anisotropy to the differences in angular rotation of the two enantiomers in the presence of the chiral selector. The term β is defined in equation 1,

$$r_S/r_R = \left[\frac{3\cos^2\beta - 1}{2}\right] \quad (1)$$

where r_S and r_R are the anisotropy values of the S and R enantiomers in the presence of the chiral selector. In this manner, nonstereoselective artifacts affect both enantiomers equally and are not reflected in the β-value. The β-values for 1,1′-bi-2-naphthol (BOH), 1,1′-bi-2-naphthyl-2,2′-diamine (BNA), 1,1′-bi-2-naphthyl-2,2′-diyl hydrogen phosphate (BNP), and Troger's base in the presence of poly-SULL are shown in Table 1. In all cases examined, significant differences in the anisotropy values were observed between the R and S enantiomers.

To evaluate the validity of this approach, micellar capillary electrophoresis (MCE) was used to determine the chiral selectivity (α) in these systems, where α is defined as[10]

$$\alpha = \frac{t_R}{t_S} \quad (2)$$

and t_R and t_S are the corrected retention times for the R and S enantiomers, respectively. This allows for an independent evaluation of the chiral selectivity of each host−guest pair that can be compared to the values predicted by the fluorescence anisotropy experiment. Chiral separations were performed under conditions similar to those of the anisotropy experiment, using the polymeric micelle as a chiral pseudostationary phase. Figure 1 shows the correlation between the selectivities predicted from the anisotropy experiment to those determined in the MCE experiment ($R^2 = 0.99$). From Figure 1 it is apparent that the β-values are linearly related to the selectivity, α, for chiral separations in MCE. Thus, the two parameters are related by

$$\alpha = m\beta + I \quad (3)$$

where m is a constant and I is the intercept. In the absence of chiral recognition, $\alpha = 1$, $\beta = 0$, and therefore, the intercept, I, must equal unity. This allows the following relationship:

$$\ln(\alpha) = \ln(m\beta + 1) \quad (4)$$

It has been well established that the difference in free energy of association between two enantiomers for a given chiral reagent is related to the logarithm of the selectivity (eq 5).[10]

$$\Delta(\Delta G)_{S,R} = -RT\ln(\alpha) \quad (5)$$

Therefore, it follows that,

$$\Delta(\Delta G)_{S,R} = -RT\ln(m\beta + 1) \quad (6)$$

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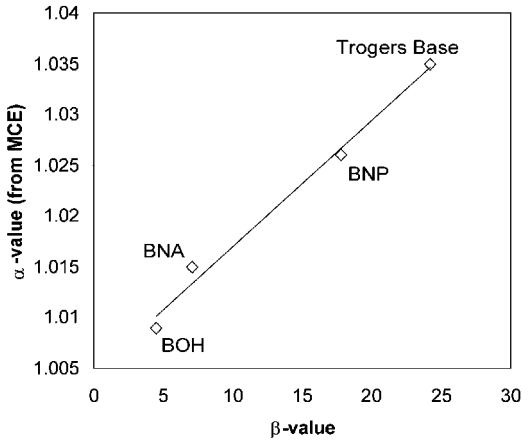

Figure 1. Plot of the correlation between the β-values derived from fluorescence anisotropy measurements and α-values obtained from MCE experiments.

Thus, the parameter, β, allows a determination of the difference in free energy of association for the enantiomeric pair. The utility of this relationship may not be readily apparent since α is readily measured from chromatographic data. In this technique, however, it is possible to determine the difference in free energy of association in systems that cannot be readily measured by chromatography, for example, the selective binding of a protein between two different enantiomers.

While there are admittedly many factors that remain to be investigated in this system, we believe that the preliminary data presented here makes a strong case for the use of fluorescence anisotropy in the investigation of chiral recognition. An exciting aspect of this research is the potentially broad applicability of the approach. The fundamental parameter being probed is the rotational diffusion of the chiral analyte; hence, the principal requirements are that the analyte possesses photoluminescent properties and that the rotational diffusion of the complex differs significantly from that of the free analyte. For this reason, the technique is potentially more broadly applicable than methods based on fluorescence quenching or enantioselective spectral pertubations. We expect that the technique will find use in the areas of chiral stationary phase development, the development of medicinal compounds, the study of enzyme/inhibitor interactions, as well as chiral selective protein interactions. The technique has already proven useful in the elucidation and optimization of chiral separation mechanisms in MCE.[11] Additionally, the technique may be useful in the determination of enantiomeric ratios. Ongoing investigations explore the exact mechanism of the enantioselective polarization, since steady-state anisotropies are affected by several parameters, most notably fluorescence quenching. In this regard, the technique of time-resolved fluorescence anisotropy is being investigated, since the technique can differentiate more precisely between the bound and free forms of the analyte. Results from these investigations will be reported in a future manuscript.

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Supporting Information Available: Experimental details of spectroscopic and separation procedures (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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