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COMMUNICATIONS

Published on Web 03/03/2004

Facile Quantification of Enantiomeric Excess *and* Concentration with Indicator-Displacement Assays: An Example in the Analyses of α-Hydroxyacids

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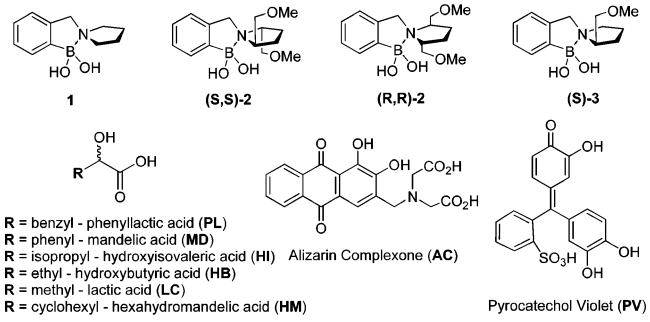
Developing enantioselective catalysts via high-throughput screening (HTS) entails determination of both reaction conversion (yield) and enantiomeric excess (ee).[1] Among the methods for ee determination,[2] the use of absorbance and fluorescence spectroscopy has been the focus of many recent studies.[3,4] In those studies, empirical calibration curves for ee are required for each different extent of conversion.[3d,e] In this study, we demonstrate that concentration and ee can be determined with two simple visible absorption measurements. The general ease of this system, and the elimination of empirical ee calibration curves for different analyte concentrations, render the technique the power to become a practical assay for the discovery of enantioselective catalysts through HTS processes.

Indicator-displacement assays (IDAs) have been used in a number of sensing applications.[5] In this study, we exploit the binding of boronic acids to α-hydroxyacids and catechols in aqueous media.[6] For example, the affinity between **1** and phenyllactic acid (PL) was determined to be 1.3×10^3 M^{-1} (Table 1) using an IDA with the catechol-containing indicators (PV and AC). Pyruvic acid, the precursor of LC through hydrogenation, does not appear to associate with **1** under such conditions. As expected, achiral receptor **1** bound both enantiomers of PL with identical affinities (Figure 1). Therefore, the total concentration of an unknown PL sample could be determined through an IDA using **1**.



By incorporating chirality into the receptor structure, the displacement of the indicator by chiral analytes was anticipated to be enantioselective. Therefore, the binding of chiral receptors (**2** and **3**) and D/L-PL was studied with an IDA also. All the receptors showed comparable affinities (Table 1) to α-hydroxyacids and PV or AC. (*S*,*S*)-**2** showed 2.8 times larger affinity to L-PL over D-PL (Figure 1), while (*R*,*R*)-**2** favored D-PL to the same extent (Table 1). Predictably, compound (*S*)-**3**, which has one less stereogenic center, displayed less discriminating power between D/L-PL. The association between (*S*,*S*)-**2** and other α-hydroxyacids was also studied (Table 2) where samples with *S*-configurations were generally favored (with the exception of LC).

When monitoring the absorbance (520 nm) of the receptor−PV complex, the different displacement profiles by D/L-PL dictate that, at a given concentration, the enantiomeric samples have distinct

Table 1. Association Constants ($K_i/10^3$ M^{-1}) of Boronic Receptors (**1**−**3**) with Indicators (PV, AC) and D/L-Phenyllactic Acids (PL)[a]

	1	(*S*,*S*)-**2**	(*R*,*R*)-**2**	(*S*)-**3**
PV	2.3	13	11	15
AC	13	63	61	57
D-PL	1.3	3.4	8.3	1.8
L-PL	1.3	9.6	3.3	2.5

[a] Measured by competitive spectrophotometry in 75% (v/v) methanolic aqueous solution buffered with 10 mM HEPES at pH 7.4 (default buffer), data with PV or AC were taken at 520 and 536 nm, respectively.

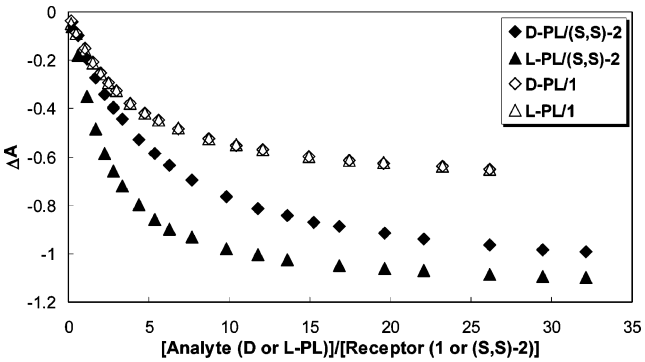


Figure 1. Absorbance change at 520 nm of PV (149 μM) and receptors (*S*,*S*)-**2** (0.510 mM), or **1** (0.575 mM) in the default buffer (footnote, Table 1) with increasing concentration of D- or L-PL (analytes).

Table 2. Association Constants (K_R, K_S) between (*S*,*S*)-**2** and α-Hydroxyacid Substrates[a]

	PL	MD	HI	HB	LC	HM
$K_R/(10^3\ M^{-1})$	3.4	2.0	4.2	3.2	4.5	4.3
$K_S/(10^3\ M^{-1})$	9.6	3.0	5.9	4.2	4.3	5.5

[a] Measured as stated in Table 1. K_R and K_S are association constants for *R*- and *S*-configured α-hydroxyacids, respectively.

UV absorbances. The difference ($\Delta\Delta A$) can be as large as 0.27. An *A* vs ee correlation at 1.5 mM analyte concentration was determined (Figure 2). The absorbance of the sample increased (filled black diamonds) when the percentage of stronger binding enantiomer (L-PL) was decreased because of less competitive binding (see Supporting Information for UV−vis spectra). When the total analyte concentration was adjusted to 3.0 mM, the overall absorption of this series of samples (blue diamonds) decreased due to more efficient displacement of the indicator, while the relative correlation between *A* and ee remained unchanged. When receptor (*S*,*S*)-**2** was replaced by its enantiomer (*R*,*R*)-**2** at a slightly different concentration, a near-mirror image *A*−ee correlation (red) was observed. Interestingly, the *A*−ee relationships were found to be curved (Figure 2), where the change in absorbance was consistently greater when the stronger-binding enantiomer was in the minority of the mixture. This is reasonable because the stronger-binding enantiomer is more dominant in the overall signal modulation.

10.1021/ja031839s CCC: $27.50

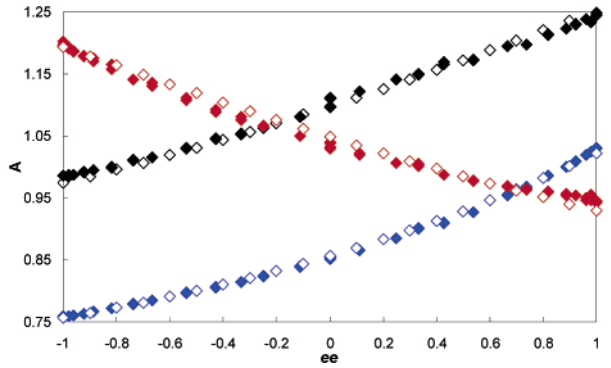


Figure 2. Absorbance change at 520 nm of PV, receptor, and analyte solutions upon increasing ee of D-PL. Black diamonds: $[I]_t = 149\ \mu M$, $[H]_t$ $[(S,S)\text{-}\mathbf{2}] = 0.51$ mM, $[G]_t = 1.50$ mM. Blue diamonds: $[I]_t = 149\ \mu M$, $[H]_t$ $[(S,S)\text{-}\mathbf{2}] = 0.51$ mM, $[G]_t = 3.00$ mM. Red diamonds: $[I]_t = 141\ \mu M$,[9] $[H]_t$ $[(R,R)\text{-}\mathbf{2}] = 0.52$ mM, $[G]_t = 1.50$ mM. Open diamonds: calculated data.

Because the behavior of all the species obey solution equilibria, the absorbance change through the variation of solution composition could be mathematically modeled.

Four interacting substances are present in solution: indicator I, chiral receptor H, and two enantiomers of the analyte G_R/G_S. Their solution species concentrations are interdependent through three equilibria: $[HG_R] = K_R[G_R][H]$, $[HG_S] = K_S[G_S][H]$, and $[HI] = K_I[I][H]$. These concentrations are related by three mass balances: $[I] + [HI] = [I]_t$, $[G_R] + [G_S] + [HG_R] + [HG_S] = [G]_t$, and $[H] + [HI] + [HG_R] + [HG_S] = [H]_t$.[7] The absorbance of the sample is given by Beer's Law (eq 1), and ee is defined in the terms of analyte concentrations (eq 2). The total of eight equations are rearranged to afford eq 3 (see Supporting Information). Parameters ϵ_I, ϵ_{HI}, and K_I are determined from a receptor/indicator binding isotherm, K_R and K_S from IDAs, $[I]_t$ and $[H]_t$ are gravimetrically determined, and the analyte total concentration $[G]_t$ is obtained from an IDA with achiral receptor **1** as previously stated. Therefore, there are only 2 variables—A and ee—in eq 3. Equation 3 is further rearranged into the standard polynomial format $PA^4 + QA^3 + RA^2 + SA + T = 0$ with the aid of the commercial software Mathematica 5,[8] where P, Q, R, S, T are all functions of ee. Therefore, by solving the 4th order polynomial equation,[8] the absorbance of the displacement cocktail is successfully correlated to the ee of the analyte. The eight experimentally determined constants (ϵ_I, ϵ_{HI}, K_I, K_R, K_S, $[I]_t$, $[H]_t$, and $[G]_t$) are input into eq 3 to generate theoretical data (open diamonds in Figure 2). The well-matched data indicate the predictive power of eq 3 for the A—ee correlations.

$$A = \epsilon_I b[I] + \epsilon_{HI} b[HI] \tag{1}$$

$$ee_R = \frac{([G_R] + [HG_R]) - ([G_S] + [HG_S])}{[G]_t} \tag{2}$$

$$\frac{A - \epsilon_I b[I]_t}{b\Delta\epsilon} + \frac{\epsilon_I b[I]_t - A}{K_I(A - \epsilon_{HI} b[I]_t)} + \frac{K_R[G]_t(1 + ee_R)(\epsilon_I b[I]_t - A)}{2[A(K_I - K_R) - b[I]_t(\epsilon_{HI}K_I - \epsilon_I K_R)]} + \frac{K_S[G]_t(1 - ee_R)(\epsilon_I b[I]_t - A)}{2[A(K_I - K_S) - b[I]_t(\epsilon_{HI}K_I - \epsilon_I K_S)]} = [H]_t \tag{3}$$

When this IDA system was put into practice to determine the concentration and ee of an α-hydroxyacid sample, two independent absorption measurements were carried out. First, the absorbance spectrum from an IDA containing the achiral receptor (**1**) and PV gave the overall concentration of the α-hydroxyacid. Second, another absorbance reading with a chiral ensemble ((*S*,*S*)-**2** and PV) was used in eq 3 to quantify the ee of the sample. This was done without generating an empirical ee calibration curve for the determined analyte concentration. The effectiveness of this system is shown in Table 3, where the total concentration and ee of three PL samples were determined. The accuracy of the overall concentration was ±10%, whereas the ee could be determined within ±20% error.[10]

Table 3. Determination of Concentration and ee of PL Samples

	concentration (actual)/mM	concentration (determined)/mM	ee (actual)	ee (determined)
1	20.0	21.5	1.00	0.98
2	26.5	28.1	−0.89	−0.71
3	28.5	31.4	−0.82	−0.68

In summary, because HTS of enantioselective catalysts demands rapid determination of both the yield and ee from a catalytic reaction, we have created a two-step analysis that utilizes an achiral and a chiral receptor in sequential IDAs. This approach is simple and practical compared to a number of reported screening assays for several reasons: it does not require substrate derivatization, it relies on a simple analytical technique (absorption spectroscopy), the production of the chiral receptors does not require lengthy syntheses, and most importantly, a mathematical analysis eliminates the need for empirical ee calibration curves for each analyte concentration.

Acknowledgment. We thank NIH (GM57306) for financial support. We are also grateful to Dr. Bo Zheng (U. of Chicago) and Mr. Frantz Andersen for helpful discussions.

Supporting Information Available: Syntheses, full derivation of eq 3, and representative absorption spectra (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(7) $[I]_t$, $[H]_t$, $[G]_t$: total indicator, receptor, and analyte concentration; HI, HG_R, HG_S: indicator-, (*R*)-analyte-, and (*S*)-analyte−receptor complex; ϵ_I, ϵ_{HI}: extinction coefficients of free and bound indicator, respectively.
(8) See http:// www.wolfram.com.
(9) In this case, the indictor concentration $[(I)]_t$ was adjusted from 153 μM (measured) to 141 μM (8%) to obtain a good fit.
(10) Judged by the displacement profiles of PL with achiral receptor **1**, the absorbance change is most sensitive when [PL]/[**1**] is between 2 and 6, which accounts for [PL] between 1 and 4 mM in the ensemble. Samples were prepared so that [PL] would fall into the effective range. The method is effective across the entire ee range and is more sensitive when the chiral receptor in use binds stronger with the *minor* enantiomer in the sample.

JA031839S