JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

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Biomimetic Chirality Sensing with Pyridoxal-5′-phosphate

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Supporting Information

ABSTRACT: Pyridoxal-5′-phosphate (PLP) is introduced to a biomimetic indicator displacement assay for simultaneous determination of the absolute configuration, enantiomeric composition and concentration of unprotected amino acids, amino alcohols and amines. The chiroptical assay is based on fast imine metathesis with a PLP aryl imine probe to capture the target compound for circular dichroism and fluorescence sensing analysis. The substrate binding yields characteristic Cotton effects that provide information about the target compound ee and the synchronous release of the indicator results in a non-enantioselective off-on fluorescence response that is independent of the enantiomeric sample composition and readily correlated to the total analyte concentration.

Pyridoxal-5′-phosphate (PLP, the active form of Vitamin B_6) is an indispensable and ubiquitous enzyme cofactor that plays a central role in transamination, racemization, elimination and many other reactions.[1] The remarkable predominance and versatility of PLP in nature has inspired the development of intriguing examples of biomimetic catalysis,[2] continuous flow chemistry,[3] combinatorial biosynthesis of complex target compounds,[4] biomolecule labeling or protein conjugation[5] and other applications.[6] Despite the myriad of pyridoxal-5′-phosphate-dependent enzymes and the variety of biological processes, the propensity of the PLP aldehyde group toward Schiff base formation with primary amino functions is a strikingly unifying leitmotif and the PLP-imine moiety is a widely encountered resting state in natural and biomimetic catalytic reactions. Despite its wide utilization and general understanding of its properties and functions, PLP has not been exploited for molecular recognition and quantitative chemosensing of chiral compounds.

The general importance of chiral amino acids, amines and amino alcohols in biological processes and asymmetric synthesis has directed increasing attention to optical methods that are capable of high-throughput screening (HTS) of the enantiomeric composition of small sample amounts.[7] In this regard, time-efficiency, cost, and compatibility with automation and micro-scale analysis are among the most important considerations whereas error margins of up to 10% ee have been considered acceptable for HTS applications.[8] The concept of covalent substrate fixation with a carefully designed aldehyde or ketone probe and subsequent enantiomeric excess (ee) determination of the bound chiral amine by UV, fluorescence or circular dichroism measurements has been successfully exploited by Kim, Hong and Chin,[9] Anslyn,[10] Pu,[11] Joyce[12] and our group.[13] Many of the chemosensors introduced to date, however, have a limited application scope and require elaborate synthesis. More importantly, the chiroptical determination of both enantiopurity and concentration of a chiral target compound remains a difficult task.[14]

We now introduce a cost-effective approach to quantitative chirality sensing using readily available PLP imines as probe (Figure 1). At the onset of this study, we rationalized that

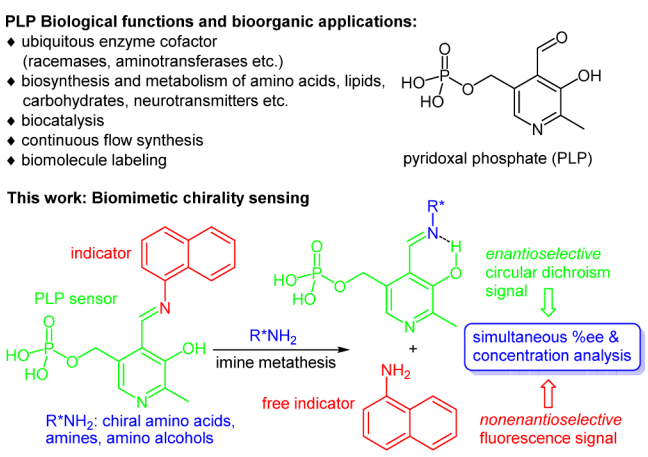


Figure 1. PLP applications and illustration of the chirality sensing concept.

condensation of PLP with an aromatic amine would afford a fast and broadly useful chirality sensor carrying an integrated indicator unit. This biomimetic sensing strategy relies on covalent binding of a chiral amino acid or another target with a primary amino group and concomitant chirality amplification onto the PLP imine scaffold to generate a circular dichroism signal that can be correlated to the ee and absolute configuration of the substrate. The binding event interconverts the chemosensor, an aromatic PLP aldimine, to a more stable aliphatic imine: a reaction that is thermodynamically favored. Because Schiff base formation and the imine exchange reaction are accelerated by the presence of the phenol and phosphate groups,[15] the use of PLP as chirality probe also addresses the need for time-efficiency in HTS applications. The imine metathesis was therefore expected to be fast, quantitative and a stoichiometric process, that is, for each bound target compound one aryl amine molecule, which we refer to as the indicator, is released. Importantly, this displacement reaction is independent of the chirality of the substrate and it consequently allows concentration analysis based on the fluorescence signal of the

Received: November 22, 2016
Published: January 27, 2017

DOI: 10.1021/jacs.6b12056
J. Am. Chem. Soc. 2017, 139, 1758−1761

free indicator regardless of the enantiopurity of the target compound as previously shown by Yu and Pu.[11c] Altogether, this biomimetic sensing approach enables one to determine both the enantiomeric excess and the total amount of amino acids, amino alcohols and amines by a simple indicator displacement assay (IDA) and fast CD and fluorescence measurements.[16]

It has been reported that free PLP and pyridoxal Schiff bases exist as complex equilibria of multiple tautomeric forms with often indistinguishable UV absorptions that are highly sensitive to the solvent polarity, pH, ionic strength, the presence of counterions, solvation effects and other environmental factors.[17] This renders efforts to use UV spectroscopy for reliable quantification of the imine metathesis impractical. Because both PLP and PLP imines typically have low quantum yields it was ruled out to accomplish this by direct fluorescence analysis and we therefore resorted to an indicator displacement assay.[18] With this in mind, we began our study with the synthesis of PLP Schiff bases exhibiting an aromatic imine moiety that would (a) be irreversibly displaced by the more nucleophilic aliphatic sensing target and (b) generate a characteristic fluorescence signal suitable for quantitative analysis upon cleavage from the PLP sensor scaffold (Figure 2 and SI).

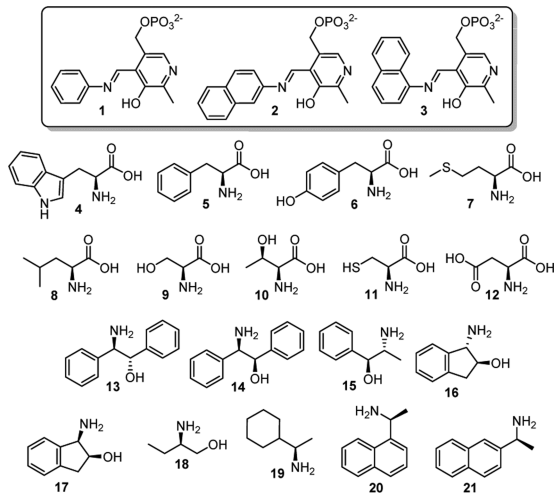


Figure 2. Structures of the PLP imine sensors **1**–**3** and 18 amino acids, amino alcohols and amines tested.

Initially, we screened solvents and bases to dissolve the PLP imine sensors and the targeted amino acids and to achieve homogeneous conditions for the imine metathesis at millimolar concentrations. We found that the desired indicator displacement occurs within a few minutes in the presence of KOH using methanol as solvent. The reaction mixtures obtained from sensor **1** and the amino acids **4**–**12** were then diluted with methanol to submillimolar concentrations and directly subjected to CD analysis. We were pleased to find characteristic Cotton effects in all cases including aliphatic substrates such as methionine (Figure 3 and SI). Essentially, the same results were obtained with the amino alcohols **13**–**18** and amines **19**–**21**.

The imine metathesis was further investigated by ^{1}H NMR and CD spectroscopy (SI). Mixing of sensor **1** and cyclohexylethylamine, **19**, in methanolic solution in the presence of stoichiometric amounts of TBAOH showed smooth displacement of the aniline moiety by the substrate within 45 s (Figure 4). The reaction coincides with a clear upfield shift of the formylimine proton from 9.39 to 9.03 ppm and of the aromatic

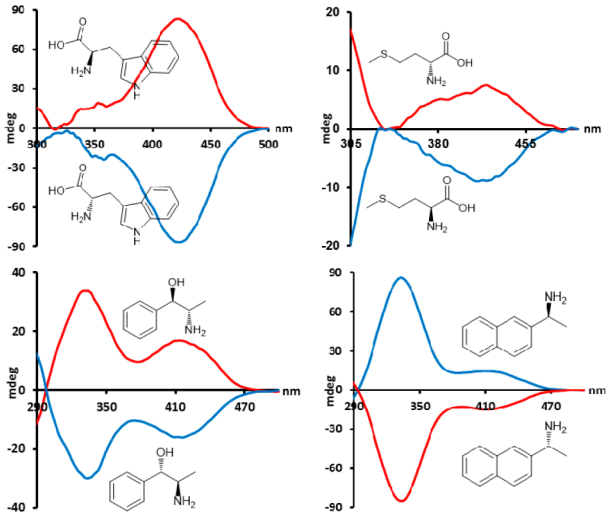


Figure 3. Chirality sensing of **4** (600 μM in MeOH), **7** (480 μM), **15** (450 μM) and **21** (360 μM) with PLP imine **1**.

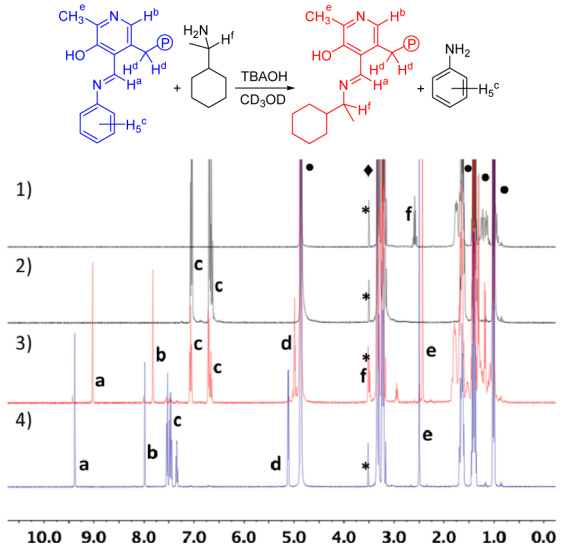


Figure 4. Proton NMR analysis of the metathesis using PLP sensor **1** and amine **19**. (1) Cyclohexylethylamine, **19**; (2) free aniline; (3) PLP-bound cyclohexylethylamine after mixing of **1** and **19** for 45 s; (4) PLP sensor **1**. All NMR spectra were recorded at 23 mM (both **1** and **19**) after addition of 1 equiv of TBAOH (1 M in MeOH) using CD_3OD as solvent. ♦ MeOH; ● TBAOH; * TBAOH-impurity.

PLP proton from 7.99 to 7.82 ppm. As expected, the cleaved indicator has upfield shifted NMR signals that are identical with free aniline and the PLP methylene and methyl groups shift from 5.12 to 4.99 ppm and 2.50 to 2.43 ppm, respectively. The PLP binding of **19** results in a downfield shift of the methine proton attached to the chiral center from 2.58 to 3.50 ppm. Importantly, the imine metathesis occurs quantitatively and without hydrolysis toward free PLP which would be clearly visible by the appearance of the PLP aldehyde signal at approximately 10.2 ppm. As a result, the indicator displacement is a perfectly stoichiometric process, i.e., the quantity of the released aniline corresponds exactly to that of the bound amine target. The generation of equimolar indicator amounts thus sets the stage for determination of the concentration of the sensing target independent of the enantiomeric composition, vide infra.

DOI: 10.1021/jacs.6b12056
J. Am. Chem. Soc. 2017, 139, 1758–1761

Having established conceptual proof for the proposed imine metathesis and chirality sensing of all 19 target compounds with the prototype sensor **1**, we continued with evaluating the possibility of quantitative ee and concentration analysis using the aminonaphthalene analogues **2** and **3**. Nonracemic tryptophan samples were applied to the indicator displacement assay with each sensor and the CD amplitudes measured at 416 nm were plotted against the enantiomeric excess. As expected, we obtained linear responses for both sensors (Figure 5 and SI).

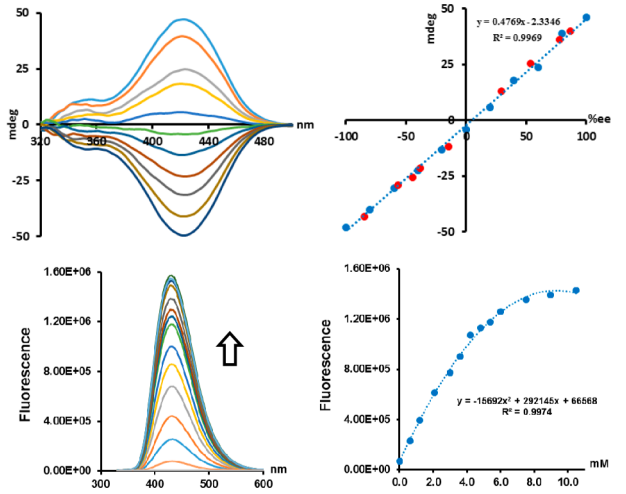
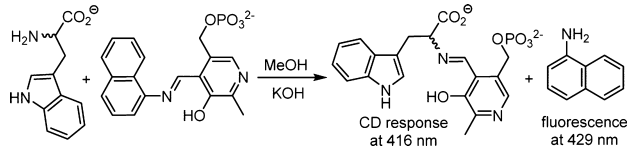


Figure 5. Top: CD effects observed with nonracemic samples of tryptophan and sensor **3** in MeOH at 480 μM (left). Linear relationship between the CD response of **3** and Trp %ee (right). The CD signals observed for Trp samples of random enantiomeric composition are shown in red. Bottom: Off–on fluorescence response to the imine metathesis and indicator release using **3** and Trp. The fluorescence spectra were obtained at 24 μM in MeOH, $\lambda_{exc.}$ 325 nm.

The known CD response of PLP sensor **3** was then used to analyze nine Trp samples with random enantiomeric composition. Four of these samples contained the D-isomer in 87%, 78%, 54% and 30% ee. We were pleased to find that the chiroptical analysis based on the indicator displacement assay with **3** gave 88%, 80%, 58% and 32% ee, respectively. The remaining five samples had the L-isomer of Trp in 14%, 38%, 44%, 56% and 85% ee and our sensing results were 20%, 41%, 49%, 56% and 86% ee. The results show that the sign of the Cotton effects provide the absolute configuration of the major Trp enantiomer, whereas the CD amplitudes measured allow calculation of accurate ee values.

As demonstrated above, the binding of enantiomeric substrates to PLP generates characteristic Cotton effects of opposite sign at high wavelengths that can be used to identify the absolute configuration and, when nonracemic samples are investigated, the intensity of the CD response yields the enantiomeric composition. Although the CD sensor response is consequently enantioselective, the release of the aminonaphthalene indicator is not and therefore suitable for total concentration analysis irrespective of the sample ee. We noticed that the PLP imines exhibit very low fluorescence signals, whereas excitation of the free indicator at 325 nm gave a strong fluorescence signal at approximately 430 nm (Figure 5). The insignificant background generated by the PLP imine sensor thus results in an off-on fluorescence response to the presence of a substrate. To test if the CD and fluorescence responses can be exploited for simultaneous ee and concentration analysis, we prepared several Trp samples of varying enantiomeric composition and amount. The samples were then employed in our indicator displacement assay and analyzed as described above using the equations from Figure 5 (Table 1). Comparison of the

Table 1. Comprehensive Chirality Sensing of Tryptophan Samples[a]



	tryptophan samples			chiroptical sensing results		
entry	abs. config.	conc. (mM)	% ee	abs. config.	conc. (mM)	% ee
1	D	4.26	41	D	4.00	49
2	L	3.06	22	L	3.05	31
3	D	2.34	54	D	2.31	61
4	D	2.04	71	D	2.19	66
5	D	5.16	93	D	5.35	90
6	/	3.60	0	/	3.21	7

[a]Circular dichroism and fluorescence sensing were performed in MeOH at submillimolar concentrations after mixing the sensor and the Trp samples for 15–20 min as described in Figure 5.

actual with the experimentally determined values show that the error margins are within the typical range that is acceptable for high-throughput screening purposes. For example, chiroptical sensing of a sample containing a 5.16 mM solution of D-Trp in 93% ee correctly confirmed that the D-form was the major enantiomer and present in 90% ee at 5.35 mM (entry 5). The PLP sensing assay can also be used for ee analysis of analyte mixtures, which is demonstrated with nonracemic **5** and **16** (SI).

In summary, we have introduced a biomimetic indicator displacement assay that exploits readily available pyridoxal phosphate derived aryl imines for simultaneous determination of the absolute configuration, enantiomeric composition and concentration of unprotected amino acids, amino alcohols and amines. The chiroptical assay is based on irreversible imine metathesis and facile circular dichroism and fluorescence measurements. The binding of the substrate by the PLP receptor generates characteristic Cotton effects that identify the major enantiomer and the sample ee. The concomitant indicator displacement results in a nonenantioselective off–on fluorescence response that is independent of the enantiomeric sample composition and readily correlated to the total analyte concentration. The sensing assay is broadly applicable: 19 chiral compounds were successfully tested, and it gives results with sufficient accuracy for HTS purposes.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b12056.

Experimental procedures, compound characterization and sensing details (PDF)

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DOI: 10.1021/jacs.6b12056
J. Am. Chem. Soc. 2017, 139, 1758–1761

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This material is based upon work supported by the NSF under CHE-1464547. P.R.B. thanks Georgetown University for a Lisa J. Raines Research Fellowship.

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DOI: 10.1021/jacs.6b12056
J. Am. Chem. Soc. 2017, 139, 1758−1761