ORGANIC LETTERS

2004
Vol. 6, No. 15
2499−2501

Using Indicator-Displacement Assays in Test Strips and To Follow Reaction Kinetics

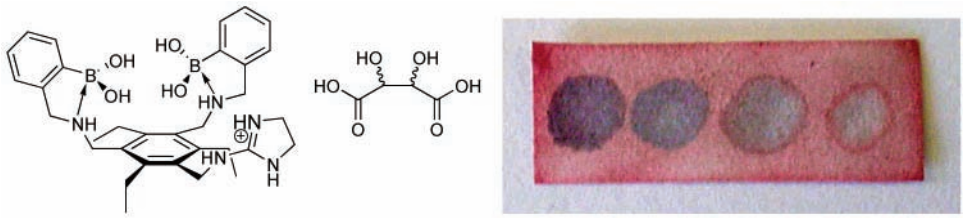
Binh T. Nguyen, Sheryl L. Wiskur, and Eric V. Anslyn*

The Department of Chemistry and Biochemistry, The University of Texas, 1 University Station A5300, Austin, Texas 78712

anslyn@ccwf.cc.utexas.edu

Received April 8, 2004

ABSTRACT



An indicator-displacement assay was used to study the kinetics of reactions that produce tartrate. The same assay was also found to be useful in developing tartrate test strips, by coating filter paper with the same receptor and indicator.

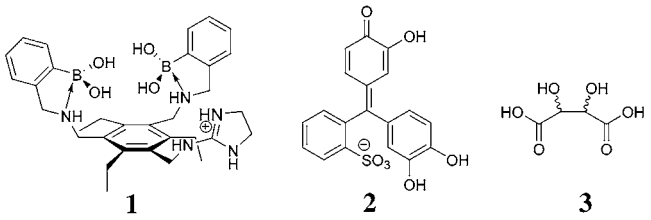
Indicator-displacement assays (IDAs) have been used widely in analytical sciences to detect various analytes. In an IDA, an indicator is first allowed to bind to a host. Then, an analyte is introduced into the system causing the displacement of the indicator from the host, which in turn modulates an optical signal.[1] The IDA offers many advantages over traditional sensing routines.[2] First, it gives a color change for a guest or host lacking a chromophore. Second, the method does not require the indicator to be covalently attached to the host. Third, because there are no covalent bonds between the host and the indicator, one can use many different indicators with the same host, and fourth, the assay works well in both organic and aqueous media. Early work using this method was successful in measuring DNA helicase activity,[3] characterizing binding of RecA protein to double-stranded DNA,[4] sensing of phosphate,[5] halides,[6] and histidine,[7] and the detection of cocaine.[8] Our group has developed a series of chemosensors that utilize an IDA to determine analytes such as gallic acid,[9] aspartate,[10] inorganic phosphate,[11] nitrate,[12] citrate,[2] glucose-6-phosphate,[2] inositol-1,4,5-triphosphate,[2] 2,3-bisphospho glycerate,[13] heparin,[14] and tartrate.[15]

Given the utility and advantages of an IDA in sensing sciences, we set out to extend the method to tracking reaction kinetics and creating test strips for "naked eye detection". Our goal was to show that an IDA can be used in these analyses by studying the kinetics of reactions that produce diastereomeric tartrates, and to qualitatively detect tartrates on test strips. Herein, we report kinetic studies of the

(1) Martinez-Manez, R.; Sancenon, F. *Chem. Rev.* **2003**, *103*, 4419.
(2) Wiskur, S. L.; Ait-Haddou, H.; Lavinge, J. J.; Anslyn, E. V. *Acc. Chem. Res.* **2001**, *34*, 963.
(3) Eggleston, A. K.; Rahim, N. A.; Kowalczykowsky, S. C. *Nucleic Acids Res.* **1996**, *24*, 1179.
(4) Zaitsev, E. N.; Kowalczykowsky, S. C. *Nucleic Acids Res.* **1998**, *26*, 650.
(5) Han, M. S.; Kim, D. H. *Angew. Chem., Int. Ed.* **2002**, *41*, 3809.
(6) Gale, P. A.; Twyman, L. J.; Handlin, C. I.; Sessler, J. L. *Chem. Commun.* **1999**, 1851
(7) Hortala, M. A.; Fabbrizzi, L.; Marcotte, N.; Stomeo, F.; Taglietti, A. *J. Am. Chem. Soc.* **2003**, *125*, 20.
(8) Stojanovic, M. N.; Landry, D. W. U.S Patent Appl. Publ. 2003.
(9) Wiskur, S. L.; Anslyn, E. V. *J. Am. Chem. Soc.* **2001**, *123*, 10109.
(10) Ait-Haddou, H.; Wiskur, S. L.; Lynch, V. M.; Anslyn, E. V. *J. Am. Chem. Soc.* **2001**, *123*, 11296.
(11) Tobey, S. L.; Anslyn, E. V. *Org. Lett.* **2003**, *5*, 2029.
(12) Niikura, K.; Bisson, A. P.; Anslyn, E. V. J. *Chem. Soc., Perkin Trans.* **1999**, 2, 1111.
(13) Best, M. D.; Anslyn, E. V. *Chem. Eur. J.* **2003**, *9*, 51.
(14) Zhong, Z.; Anslyn, E. V. *J. Am. Chem. Soc.* **2002**, *124*, 9014.
(15) Lavinge, J. J.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **1999**, *38*, 3666.

10.1021/ol0493599 CCC: $27.50 © 2004 American Chemical Society
Published on Web 06/26/2004

hydroxylation of maleic acid and fumaric acid in water to create tartrate, as well as the hydrolysis of D-dimethyl tartrate. We further show that an IDA is easily adapted to test strips.



1 **2** **3**

The design of host **1** has been reported.[9] Previous studies showed that boronic acids form reversible bonds with 1,2-diols and α-hydroxy carboxylate,[16] and guanidinium groups bind carboxylates through H-bonds or charge-pairing interactions.[17,18] Further, on the basis of our previous studies, we knew that upon addition of **1** to a solution of **2** (0.05 mM, 25% water in methanol (v/v), 20 mM HEPES, pH 7.4) the λ_{max} of the indicator shifts from 441 nm to that of the indicator−host complex. Addition of **3** to the solution of the **1**:**2** complex causes the λ_{max} to shift back to 441 nm. The binding constants between **1** and **2** and between **1** and *d*,*l*-**3** are known to be $K_{HI} = 6.1 \times 10^4\ M^{-1}$ and $K_{HT} = 1.4 \times 10^5\ M^{-1}$, respectively.[9,19]

To follow the kinetics of tartrate formation, one must relate the free indicator concentration to tartrate concentration. In an IDA, the change of absorbance of the indicator is not directly proportional to tartrate formation because of the equilibria in eq 1, where H is the host, I is the indicator, and T is tartrate. We solved for the relationship between indicator concentration and tartrate concentration (eq 2). [HI] and [I] can be related to absorbance changes via eqs 3 and 4, where HI is host−indicator complex, HT is the host−tartrate complex, $[H]_0$ is the total concentration of the host, $[I]_0$ is the total concentration of the indicator, A_I is the absorbance of free indicator, A is measured absorbance, and A_{HI} is absorbance of the host-indicator complex. Last, [T] is used in eq 5 to create the standard first order kinetics plot, where $[S]_0$ is the total concentration of the reactant, t is time, and k is the reaction rate constant.

$$HI + T \rightleftharpoons HT + I \tag{1}$$

$$[T]\frac{[H]_0K_{HI}[I] - [HI] - K_{HI}[I][HI]}{K_{HT}[HI]} \tag{2}$$

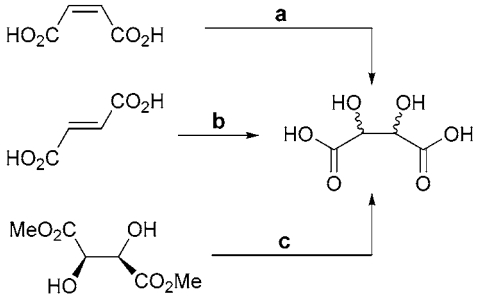
$$[HI] = [I]_0\frac{A_I - A}{A_I - A_{HI}} \tag{3}$$

$$[I]_0 = [I] + [HI] \tag{4}$$

$$-\ln\frac{[S]_o - [T]}{[S]_o} = kt \tag{5}$$

We applied the mathematic derivation above to study the kinetics of the dihydroxylation of maleic acid and fumaric acid in water, and the hydrolysis of *D*-dimethyl tartrate (Scheme 1). In the hydroxylation of maleic acid and fumaric acid, 2 equiv of NaOH were used to bring the pH of the solution near 7 so that when adding an aliquot (2 μL) of the reaction mixture to the solution of the **1**:**2** complex in buffer the overall pH of the solution would not be perturbed. Typically, the reaction kinetics were followed by adding 2 μL of the reaction mixture into 1 mL of a solution of the **1**:**2** complex (0.25 mM **1**, 0.05 mM **2**, 25% water in methanol (v/v), 20 mM HEPES, pH 7.4) at each time interval, and the absorbance was measured. For each interval of time, a fresh 1 mL of the solution of the **1**:**2** complex was used because for this particular reaction, the OsO_4 would dihydroxylate the indicator after several minutes. The UV−vis spectra and plots of kinetics are shown in Figure 1. Least-squares fitting of the data to eq 5 gives rate constants for the dihydroxylation of maleic acid and fumaric acid catalyzed by osmium tetraoxide in water as $3 \times 10^{-4}\ h^{-1}$ and $6 \times 10^{-4}\ min^{-1}$ respectively. The rate constant for the hydrolysis of D-dimethyl tartrate was $3 \times 10^{-4}\ h^{-1}$.

Scheme 1. Formation of Tartrate[a]



[a] Conditions: (**a**, **b**) 0.5 M, NaOH (2 equiv), $NaClO_3$ (1.6 equiv), water, 4% w/w OsO_4 in water (0.4% mol equiv), 50 °C; (**c**) 0.2 M, water, pH 2, 100 °C.

Besides applying the IDA to reaction kinetics, we also developed a tartrate test strip that produced colors with different intensities upon applying different tartrate concentrations. The test strip was prepared by adding 1−2 mL of buffer (20 mM HEPES, 25% water in methanol (v/v), pH 7.4) to a 3 cm × 1 cm filter paper (Fisher, Q8, cat. no. 09-790F). After drying in air, 1 mL of the **1**:**2** complex (0.2 mM **1**, 0.2 mM **2**) was allowed to coat the paper, which was placed on a watch glass. The solution was absorbed by the paper, and the paper was dried in air. Using capillary tubes, four different solutions of tartaric acids (1, 0.5, 0.1, and 0.01 M at pH 7.2) were spotted on the paper. The spots turned dark blue and the intensity of the color decreased as the concentration of tartrate decreased (Figure 2).

To exclude the possibility of residual metal in the paper matrix, which may influence the color change, a control test strip was made by pretreating the paper with a solution of 20 mM EDTA and washing with the same buffer, and the

(16) Wulff, G. *Pure Appl. Chem.* **1982**, *54*, 2093.
(17) Linton, B. R.; Goodman, M. S.; Fan, E.; Van Arman, S. A.; Hamilton, A. D. *J. Org. Chem.* **2001**, *66*, 7313.
(18) Berger, M.; Schmidtchen, F. P. *J. Am. Chem. Soc.* **1999**, *121*, 9986.
(19) Wiskur, S. L. Ph.D. Thesis. The University of Texas, Austin, Texas 2003.

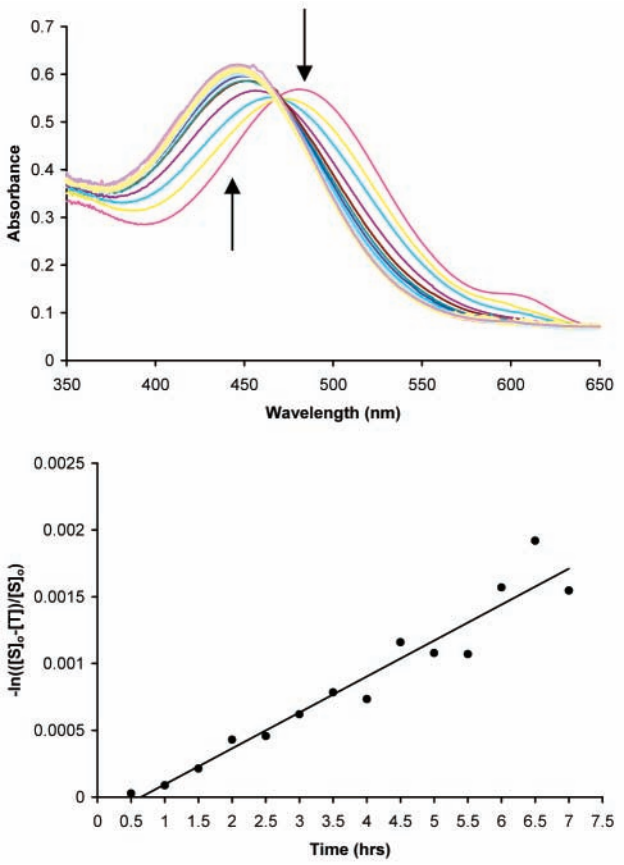


Figure 1. UV−vis spectrum for the IDA and kinetic plot for the analysis of the dihydroxylation of maleic acid.

procedure above was repeated. The same color change was observed. When using the same coating conditions with the addition of buffer or water, no color change was observed. Also, a test strip coated with the indicator alone (0.2 mM, 20 mM HEPES, 25% water in methanol (v/v), pH 7.4) tested with the same tartrate solutions produced no color change. These results suggest that the IDA is suitable for making test strips for visual detection of specific chemicals that have no chromophore of their own.

In conclusion, we have found two new applications for indicator-displacement assays. It is now possible to study reaction kinetics, and we specifically followed reactions that create tartrate. Further, the first test strip based upon an IDA was reported. The results of this research open a window of opportunity for the future of IDAs. This very simple method could be used to follow the kinetics of simple reactions and detect other natural or synthetic products using colorimetric test strips.

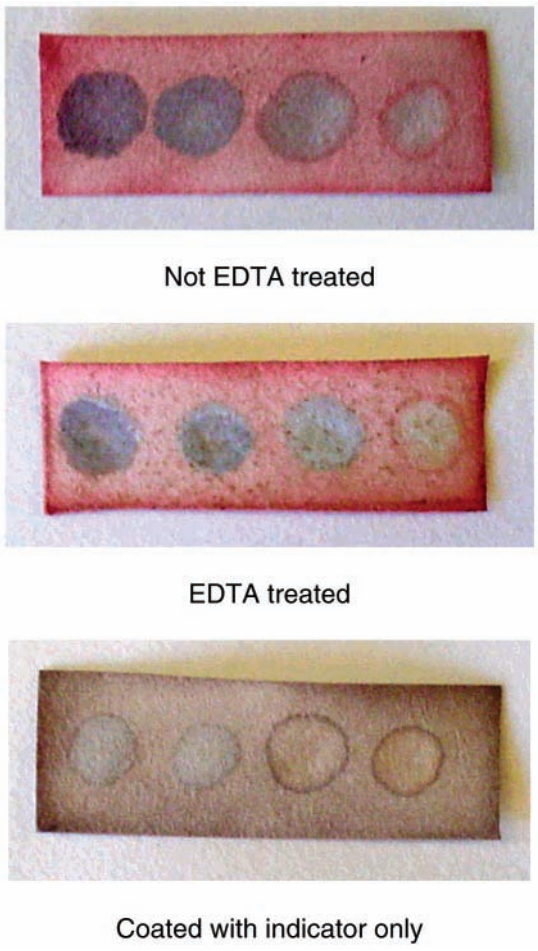


Figure 2. Test strips tested with 1.0, 0.5, 0.1, and 0.01 M tartrate at pH 7.2.

Acknowledgment. We gratefully acknowledge support for this project from the Welch Foundation and the Beckman Center for array sensors.

Supporting Information Available: Full derivation of equations. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0493599