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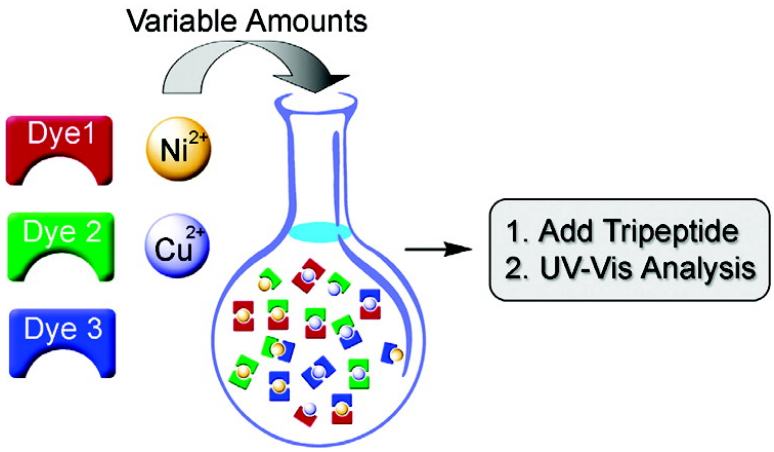

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Easy to Optimize: Dynamic Combinatorial Libraries of Metal−Dye Complexes as Flexible Sensors for Tripeptides

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A dynamic combinatorial library (DCL) of dye complexes of Cu^{2+} and Ni^{2+} was used to sense the sequence isomeric tripeptides His-Gly-Gly, Gly-His-Gly, and Gly-Gly-His. The DCL sensor was obtained by mixing buffered aqueous solution of three commercially available dyes together with $CuCl_2$ and $NiCl_2$. The addition of the peptide analytes resulted in characteristic changes in the UV−vis spectrum of the mixture, which allowed the identification of the peptide. Due to the modular nature of the sensor, it was possible to optimize the sensor by variation of the amounts and ratio of its constituent building blocks. The composition of the best sensor was found to vary substantially, depending on the sensing problem to be addressed. Whereas a Cu^{2+}/Ni^{2+} ratio of 1:3 gave the best differentiation for Gly-His-Gly and Gly-Gly-His, a sensor containing exclusively Cu^{2+} was found to provide the best discrimination between His-Gly-Gly and Gly-Gly-His.

Introduction

Initiated by work of the groups of Lehn,[1−3] Sanders,[4−7] and others[8−13] in the mid-1990s, dynamic combinatorial chemistry (DCC) has become an active field of current research.[14−16] DCC strategies have successfully been employed for the discovery of new enzyme inhibitors,[17−19] receptors,[20] and catalysts,[21] as well as for the synthesis of novel materials, such as responsive gels and polymers.[22] The adaptive behavior of dynamic combinatorial libraries (DCLs) has received particular attention in this context. DCLs are formed by combinatorial assembly of molecular building blocks via reversible covalent or noncovalent bonds. They represent chemical networks,[23] the composition of which can be influenced by external stimuli. Upon addition of a target molecule that selectively interacts with some members of the library, a reequilibration may occur. This adaptation can be used to identify library members with a high affinity for the respective target.[24−28]

We have recently reported the utilization of a DCLs as a colorimetric sensor.[29] The basic idea was that an analyte-induced adaptation of a DCL can be used to identify the respective analyte, given that the library is composed of compounds of different colors. For such a sensor, the information about the sample is distributed over the entire UV−vis spectrum, which represents a "fingerprint" for the analyte. In this regard, a DCL sensor is related to colorimetric sensor arrays.[30−32] Here, the sample is identified by an analysis of several nonspecific sensors, the ensemble of which provides a characteristic "fingerprint". But contrary to sensor arrays with independent sensor units, a DCL sensor consists of compounds that are connected by exchange reactions. Furthermore, the various sensors of an array have to be addressed separately, whereas for a colorimetric DCL sensor, a single UV−vis measurement is sufficient as the read-out.

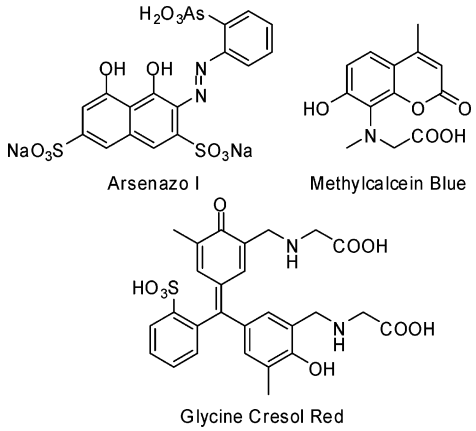

Figure 1. Chemical structures of the three dyes that were employed to build a DCL of metal−dye complexes.

As a proof of principle, we had generated a DCL of metal−dye complexes by combination of three metal-binding dyes, Arsenazo I, Methylcalcein Blue, and Glycine Cresol Red (Figure 1), with $CuCl_2$ and $NiCl_2$ in buffered aqueous solution.

The resulting complex mixture consisted mainly of 1:1 and 2:1 complexes (homo- and heteroleptic) that were in a dynamic equilibrium with each other, as evidenced by UV−vis measurements.[29] Dipeptides were used as analytes. They were found to undergo ligand exchange reactions with the metal−dye complexes. The addition of a dipeptide to the DCL sensor, therefore, resulted in a partial liberation of dyes accompanied by a reequilibration of the remaining metal−dye complexes. Since the free dyes and the various metal−

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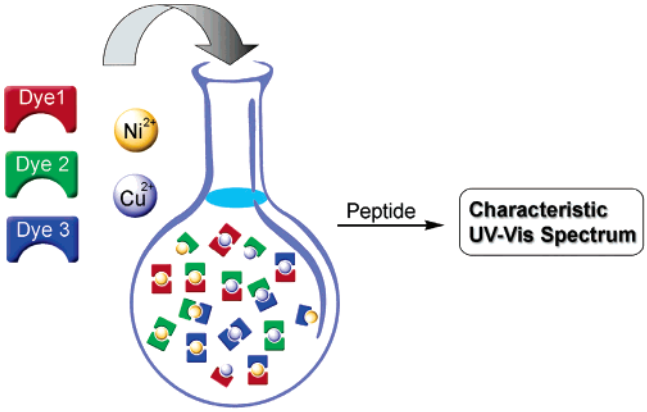

Figure 2. The addition of a peptide to a dynamic combinatorial library of metal−dye complexes leads to a reequilibration of the library and, thus, to characteristic changes in the UV−vis spectrum.

dye complexes have different colors, a change in the UV−vis spectrum was observed. This change was characteristic for each dipeptide, which allowed their identification (Figure 2).[29]

The sensing of peptides with a DCL of metal−dye complexes is experimentally very simple because all that is required is to dissolve commercially available dyes together with transition metal salts in a buffer and to record a UV−vis spectrum. In the following, we demonstrate that the approach has another major advantage: its inherent flexibility. A DCL sensor is generated by self-assembly of multiple subunits. This allows one to rapidly optimize the system for a specific sensing problem by variation of the amounts and the relative ratios of the constituent building blocks in a combinatorial fashion. For the analysis of sequence-isomeric tripeptides, we will show that the best sensor composition is strongly dependent on the analytical problem that is addressed. With the optimized sensor, it is possible to discriminate mixtures of tripeptides from the pure samples and to obtain quantitative information.

Experimental Section

Chemicals and Procedures. The following chemicals were purchased and used as received: Arsenazo I (Lancaster); Methylcalcein Blue (Sigma); Glycine Cresol Red and $CuCl_2 \cdot 2H_2O$ (Fluka); $NiCl_2 \cdot 6H_2O$ (Strem Chemicals); CHES buffer (Acros); and His-Gly-Gly, Gly-His-Gly, and Gly-Gly-His (Bachem). Stock solutions of the dyes (1.0 mM), the metal salts (10.0 mM), the peptides (5.0 mM), and CHES buffer (pH 8.4, 200 mM) were prepared in bidistilled water. All UV−vis spectra were recorded on a Lambda 40 spectrometer (Perkin-Elmer). The DCL sensors were prepared by mixing appropriate amounts of stock solutions of the three dyes, the metal salts, and the buffer (final concentration: [Arsenazo I] = [Methylcalcein Blue] = [Glycine Cresol Red] = 75 μM, $[M^{2+}]_{total}$ = 80−320 μM, 100 mM CHES buffer, pH 8.4). After addition of the respective peptide, the solutions were equilibrated for 2 h at room temperature, and the UV−vis spectra (350−700 nm; 1 nm increments) were recorded.

Data Analysis. For the optimization of the DCL sensors, five independent measurements were performed for each DCL−peptide combination. To quantify the ability of the respective sensor to differentiate the two peptides, the area between the two UV−vis curves was calculated as $\sum_{\lambda=350nm}^{\lambda=700nm} |A1(\lambda) - A2(\lambda)|\Delta\lambda$, with $A1(\lambda)$ and $A2(\lambda)$ being the averaged absorption values A measured for the two peptides 1 and 2 at the wavelength λ and $\Delta\lambda$ being 1 nm. The linear discriminant analysis (LDA) was performed with the commercially available statistical software SYSTAT (version 11.0). For each measurement, 351 data points (absorbance values in the region λ = 350−700 nm) were used as an input. To determine the wavelengths, which contribute most to the differentiation, a variable selection algorithm was utilized. The selected variables were then used to calculate the classification functions and to generate the score plots.

Results and Discussion

Chemosensors, which are able to sense small peptides in aqueous solution, are the objects of extensive studies.[33−37] These investigations are motivated by the fact that short peptides are involved in many biological processes, and analytical tools to selectively detect these compounds are, thus, of high interest. For our experiments, we focused on three different analytes: the sequence isomers His-Gly-Gly (**1**), Gly-His-Gly (**2**), and Gly-Gly-His (**3**). Our initial goal was to optimize a DCL sensor for the discrimination of the analytes **1** and **3** and of **2** and **3**. As building blocks, we used again the three dyes Arsenazo I, Methylcalcein Blue, and Glycine Cresol Red together with $CuCl_2$ and $NiCl_2$. The peptides **1**, **2**, and **3** are known to coordinate to Cu^{2+} and Ni^{2+} in aqueous solution.[38] Depending on the nature of the peptide, the metal ion, and the pH, complexes with different stoichiometries are formed. It was, thus, expected that the addition of the tripeptides to a mixture of metal−dye complexes would result in a partial liberation of dyes accompanied by a reequilibration of the remaining metal−dye complexes and, hence, in changes in the UV−vis spectra.

For the screening, 20 different sensors were generated for each analyte pair by variation of the total (80, 160, 240, and 320 μM), and the relative metal concentration using a constant dye concentration of [Methylcalcein Blue] = [Arsenazo I] = [Glycine Cresol Red] = 75 μM in buffered aqueous solution (100 mM CHES, pH 8.4). Five independent measurements were performed for each DCL−peptide combination ([peptide] = 1.00 mM). The analytes gave rise to characteristic UV−vis spectra, which were dependent on the DCL composition. To approximate the ability of the respective DCL sensor to discriminate between two peptides, we calculated the area between the UV−vis curves obtained for the two analytes. The data for the peptides **2** and **3** using a DCL with a metal concentration of $[Ni^{2+}]$ = 240 μM and $[Cu^{2+}]$ = 80 μM are shown in Figure 3. The overall results of the screening are depicted in Figure 4.

For the discrimination of **2** and **3** as well as of **1** and **3**, a high total metal concentration of 320 μM was found to be advantageous. Interesting differences, however, were observed for the best Cu^{2+}/Ni^{2+} ratio. For the analyte pair **2** and **3**, a mixture of 25% Cu^{2+} and 75% Ni^{2+} resulted in the largest difference in the UV−vis spectra. For the analyte pair **1** and **3**, on the other hand, a sensor containing exclusively copper gave the best results. This demonstrated

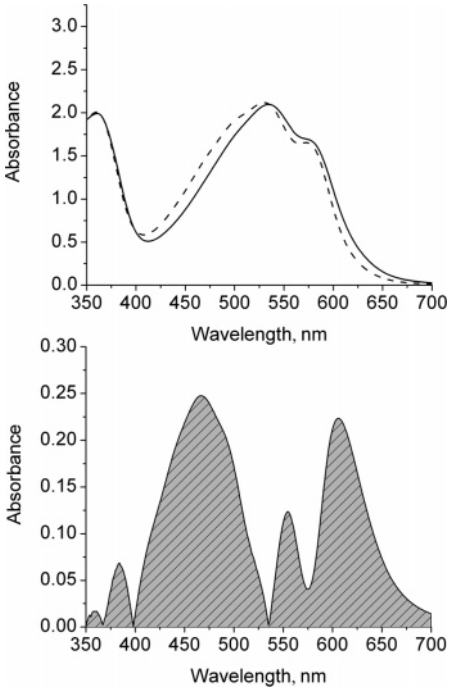

Figure 3. Top: UV−vis spectra of a DCL of metal−dye complexes after addition of the tripeptides **2** (solid line) or **3** (dashed line); Bottom: absolute difference between the two curves. Conditions: [peptide] = 1.00 mM; [Methylcalcein Blue] = [Arsenazo I] = [Glycine Cresol Red] = 75 μM, [Ni^{2+}] = 240 μM, [Cu^{2+}] = 80 μM, 100 mM CHES buffer, pH 8.4. The data represent averaged values from 5 independent measurements.

that the optimal sensor composition can vary substantially, even for closely related analytes.

The small screening of 20 different sensors was not expected to result in the identification of the overall best sensor composition for a given analyte pair, since other important parameters, such as the total or relative dye concentration, were not varied. Nevertheless, our best sensors already possess a remarkable analytical power, as demonstrated by the following results.

An interesting analytical problem is the discrimination of pure samples from mixtures. To address this issue, we used the optimized sensor containing 75% Ni^{2+} (240 μM) and 25% Cu^{2+} (80 μM) for the analyte pair **2** and **3**. Five different samples containing either the pure peptides **2** and **3** or mixtures of the two (**2**/**3** = 3:1, 1:1, and 1:3) were analyzed. The total peptide concentration in all cases was 1.00 mM. Five independent UV−vis measurements were performed for each sample, and the resulting data were then classified by a linear discriminant analysis. Each spectrum consisted of 351 data points (350−700 nm; $\Delta\lambda$ = 1 nm), most of which are linearly dependent and, hence, contain no unique information about the analyte. We therefore employed a selection algorithm to choose the variables (wavelengths), which contribute the most to the differentiation between analytes. The selected variables were then used to calculate the classification functions and to generate the score plots, which show the clustering of the data (Figure 5).

The analysis evidenced that the sensor response permits the identification of the respective sample without any misclassification. It was, thus, possible to discriminate Gly-His-Gly (**2**) from Gly-Gly-His (**3**) and from mixtures of the

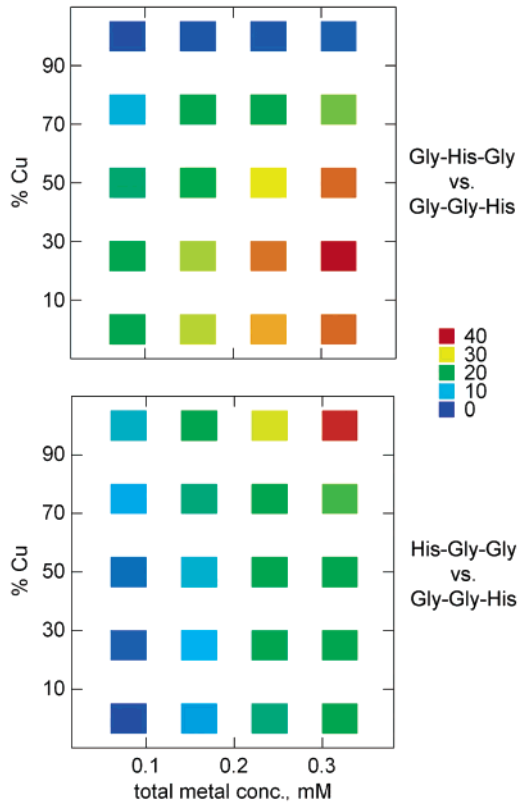

Figure 4. The ability of a DCL sensor to discriminate between the sequence isomers **2** and **3** (top) and **1** and **3** (bottom) as a function of the total metal concentration and the Cu^{2+}/Ni^{2+} ratio. The color coding indicates the differentiation, which was achieved for the respective sensor composition. The data (arbitrary units) are based on five independent measurements; the errors are <2 units.

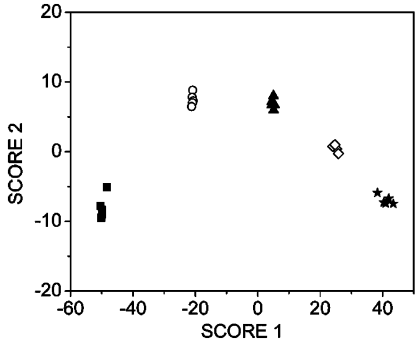

Figure 5. Two-dimensional LDA score plot for the analysis of the tripeptide **2** (★), **3** (■), and of mixtures of the two peptides at the following ratios: **2**/**3** = 1:3 (○), **2**/**3** = 1:1 (▲), and **2**/**3** = 3:1 (◇).

two by a simple UV−vis measurement. From Figure 5, it is also apparent that the resulting data can be used as a "calibration curve" to estimate the **2**/**3** ratio of samples with unknown composition.

In a second set of experiments, we investigated the possibility to simultaneously identify and quantify samples containing the peptide either **2** or **3**. For each peptide, four samples with a total concentration of 0.25, 0.50, 0.75, and 1.00 mM were analyzed by five independent UV−vis measurements. The data were treated as described above. From the resulting LDA score plot (Figure 6), it is evident that the DCL sensor is, indeed, able to provide information

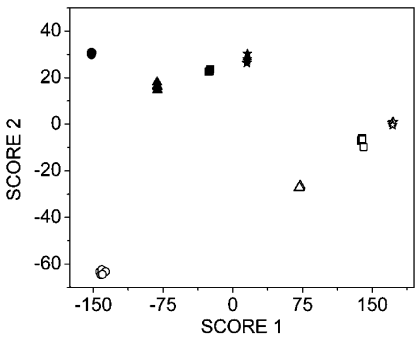

Figure 6. Two-dimensional LDA score plot for the analysis of the tripeptides **2** (filled symbols) and **3** (closed symbols) at various concentrations (0.25 mM, circles; 0.50 mM, triangles; 0.75 mM, squares; and 1.00 mM, stars).

about the nature *and* the amount of the respective peptide. The accuracy of a quantitative determination is estimated to be ±10 μM.

Conclusion

DCL sensors are by definition complex chemical systems. Consequently, it is difficult to predict the sensor response for a given analyte. The lack of control is compensated by the fact that DCL sensors can easily be modified, and thus optimized, by variation of the nature, the amounts, and the relative ratios of the constituent building blocks. This key advantage is demonstrated by the results described above. A DCL sensor consisting of three dyes and two metal salts was optimized for the differentiation of sequence-isomeric tripeptides by screening the response of 20 sensors with a variable composition. The identity of the best sensor was found to depend on the problem that was addressed. The analytical power of the optimized sensor was sufficient to differentiate aqueous solutions containing a variable ratio of the tripeptides Gly-His-Gly and Gly-Gly-His and to obtain quantitative information about these analytes. With regard to potential applications, it should be noted that the experimental setup for a colorimetric DCL sensor as described above is extremely simple: all that is required is to mix commercially available dyes with transition metal salts in a buffer. This is in contrast to other chemosensors for small peptides, the preparation of which often requires substantial synthetic efforts.[33−36]

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