

A Chemosensor Array for the Colorimetric Identification of 20 Natural Amino Acids

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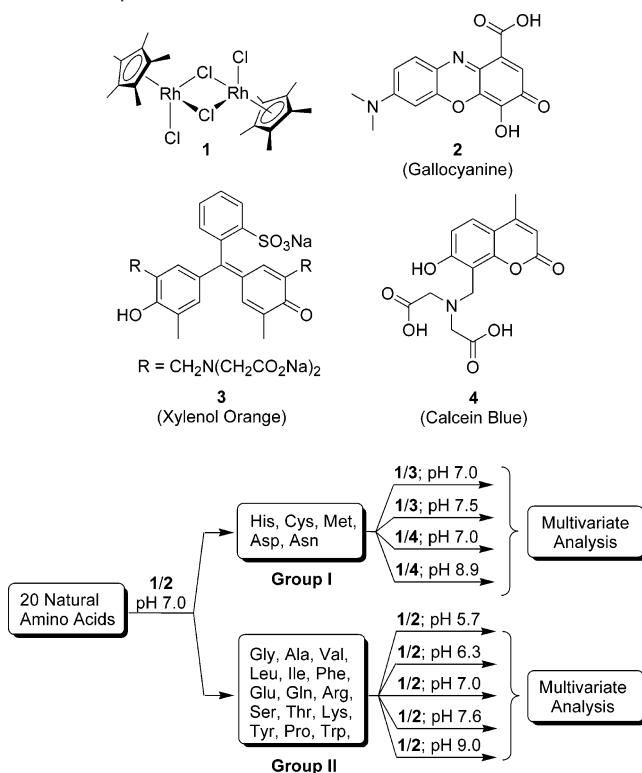
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Indicator displacement assays (IDAs) have been successfully applied to sense a variety of analytes such as citrate,¹ heparin,² phosphates,³ carbonate,⁴ amino acids,⁵ and peptides^{5c,6} among others.⁷ The basis of IDAs are chemosensors in which the receptor units are connected via noncovalent interactions to signaling units (indicators). Upon addition of a substrate, the indicators are replaced, resulting in a change of their optical properties. This type of supramolecular approach toward sensing has the advantage that it is very simple to implement. Furthermore, it is possible to modulate the sensitivity as well as the selectivity of the assay by variation of the receptor–indicator ratio⁸ or the sensing conditions (e.g., pH, see below). Thus far, the focus has been on constructing highly selective IDAs using carefully designed receptor units with high specificity for a certain analyte.⁷ The utilization of IDAs for multianalyte sensing⁹ or for array technologies^{10,11} is largely unexplored. In the following we describe an IDA-based sensor array, in which the respective sensors are assembled from commercially available building blocks. This array allows the differentiation of 20 natural amino acids with high fidelity using UV/vis spectroscopy in combination with a multivariate analysis.

Over the last 5 years, a number of IDA-based sensors for amino acids have been described.⁵ Selectivity was mainly achieved by using receptors which are able to recognize a certain functional group of an amino acid side chain (e.g., imidazole of His, thiol of Cys, or carboxylate of Asp or Glu). To distinguish simple, nonfunctionalized amino acids such as Ala or Val, however, a single-sensor approach would be difficult to realize because of the lack of sufficiently specific receptors. We therefore decided to investigate whether an array-based approach would be suitable for achieving the required selectivity.^{12,13} The organometallic Cp*Rh complex **1** was employed as the receptor unit for our IDAs (Scheme 1). This complex is soluble in water, not sensitive to air, displays relatively fast exchange kinetics for the three facial coordination sites opposite to the Cp* ligand, and shows a high affinity for amino acids and peptides.¹⁴ A first application of **1** in IDAs for His- and Met-containing peptides has recently been described.⁶ As the indicators, the dyes gallocyanine (**2**), xylénol orange (**3**), and calcein blue (**4**) were employed. These dyes bind to the Cp*Rh complex with a strong change in color and with a variable affinity in the low micromolar range (see Supporting Information). The binding constants of the amino acids to the Cp*Rh complex were found to be in the same range as those of the dyes, resulting in a real competition situation for at least one dye–amino acid combination. A key component of our sensor array was the utilization of a variable pH. The pH was expected to change the selectivity of the IDAs because the relative binding affinity of a given dye–amino acid pair depends on the pH.¹⁵

The procedure for the colorimetric identification of 20 natural amino acids using an IDA-based assay is summarized in Scheme 1. First, the UV/vis response at 750 nm of an IDA composed of **1**

Scheme 1. Colorimetric Identification of 20 Natural Amino Acids Using IDA Arrays Composed of Receptor **1** and the Indicators **2–4** at Different pHs



(50 μM) and gallocyanine (**2**) (100 μM) was used to classify the amino acids (750 μM) into a high- and a low-affinity group. The high-affinity group consists of His, Cys, Met, Asp, and Asn for which a nearly quantitative replacement of the dye **2** from the rhodium complex was observed ($\Delta A(750 \text{ nm}) < 0.06$).¹⁶ The remaining 15 amino acids lead to a partial replacement of the dye ($0.55 > \Delta A(750 \text{ nm}) > 0.06$) and constitute the second group. After this separation, each member of the first group was analyzed by four IDAs (two different dyes, **3** and **4**, and two different pH values), and each member of the second group was analyzed by five IDAs (indicator **2** at five different pH values).¹⁷ In each case, the analysis was repeated 12 times with all data being gathered in a highly parallel fashion using a microplate reader.

The data for three selected amino acids of group II (Ala, Phe, Pro) are shown in Figure 1. Despite the fact that these amino acids do not possess additional functional groups on their side chains, a unique absorption pattern was obtained. To evaluate whether this “fingerprint” can be used for the identification of the amino acids, a linear discriminant analysis (LDA) was performed.¹⁸

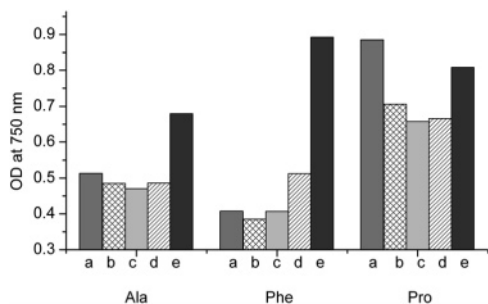


Figure 1. Absorbance at 750 nm for mixtures containing the dye **2** (100 μM), the receptor **1** (50 μM), and the respective amino acids (750 μM) determined at five different pH values: 5.7 (a), 6.3 (b), 7.0 (c), 7.6 (d), and 9.0 (e) (MES, phosphate or CHES buffer, 85 mM).

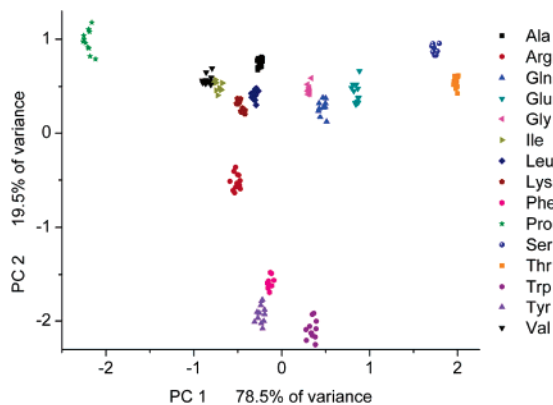


Figure 2. Score plot for the identification of the amino acids of group II.

The predictive ability of our sensor array was determined by a cross-validation routine, in which randomly one measurement was omitted. The remaining data were used as the training set for the LDA, and the omitted observation was classified. The data of group I and II were treated separately. For group I, all data were assigned accurately, and for group II, 1 misclassification out of 180 observations was obtained (99.4% accuracy). If only 50% of the data were used as the training set, group I was still assigned with 100% accuracy. For group II, 174 out of 180 observations were classified correctly (96.7% accuracy). The amino acids, which gave rise to these occasional mismatches were valine and isoleucine.

To visualize the clustering of the data, a principal component analysis (PCA) was performed.¹⁸ The results for the amino acids of group II are depicted in Figure 2. The data appear in well-separated groups with the only overlap found for valine and isoleucine. To some extent, chemical and structural similarities are reflected by the relative position of the group. The data of the hydroxy amino acids serine and threonine, for example, are positioned in proximity to each other as well as the data for the aromatic amino acids phenylalanine, tyrosine, and tryptophan. The only cyclic amino acid, proline, is well separated from the rest.

The results described above demonstrate the analytical power of IDAs performed in an array format. Closely related analytes such as leucine and isoleucine are clearly distinguishable, a result which would be very difficult to achieve with a classical one sensor–one analyte approach. The IDA array used for our analysis is very easy

to build because the only requirement is mixing of commercially available building blocks at different pHs. It appears likely that similar assays can be constructed for the analysis of more complex analytes such as peptides and proteins. Research along these lines is currently being pursued in our laboratory.

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Supporting Information Available: UV/visible titration data for the reaction of receptor **1** with the indicators **2–4**, experimental procedures for the IDAs, a description of the data analysis, and a score plot for the amino acids of group I. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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- (15) A behavior of this kind is expected for ligands, the metal-binding groups of which have a different pK_a .
- (16) $\Delta A = A(1 + 2 + \text{amino acid}) - A(2)$.
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