Chemosensors

An Organometallic Chemosensor for the Sequence-Selective Detection of Histidine- and Methionine-Containing Peptides in Water at Neutral pH**

*Andrey Buryak and Kay Severin**

A synthetic receptor, which is bound through noncovalent interactions to an indicator, can function as a chemosensor. The fundamental requirement is that the displacement of the indicator by an analyte results in a change in its optical properties.[1] An indicator-displacement assay (IDA) of this kind has been used to detect analytes such as citrate,[2] tartrate,[3] gallic acid,[4] heparin,[5] phosphates,[6] carbonate,[7] and amino acids.[8] Relative to "classical" chemosensors with integrated binding and signaling components, IDAs have two major advantages: 1) they are easy to generate because the signaling unit is attached by noncovalent interactions, and 2) the physical properties of the indicator (color, affinity for the host, solubility) as well as the indicator–receptor ratio[9] can be varied according to specific needs.

To attach the indicator to the binding site of the host, electrostatic interactions, hydrogen bonding, and/or metal–ligand interactions have been employed. The latter mode of attachment has the advantage that it is able to provide sufficiently high association constants, even in polar solvents such as water. So far, 3d transition-metal ions have been used almost exclusively for this purpose.[10] At first glance, 4d and 5d transition metals appear to be less suited because they generally display slower exchange kinetics. On the other hand, they may show very high binding constants which would allow the detection of analytes at low concentrations. Furthermore, they preferentially bind to ligands with "soft" donor groups (e.g. amines) which may be of interest for certain analytes. Herein, we demonstrate that the combination of an organometallic Cp^*Rh^{III} complex **1** with the indicator azophloxine (**2**) comprises an IDA, which allows the selective detection of histidine- and methionine-containing peptides.

In continuation of our studies of organometallic receptors,[11,12] we have investigated whether half-sandwich complexes of rhodium(III) can be reversibly attached to indicators to build a sensing ensemble as described above. The Cp*Rh complex **1** appeared to be well suited for this purpose because it is soluble in water and because the exchange kinetics for the three facial coordination sites opposite to the π ligand are fast.[13] The screening of a number of commercially available indicators showed that the dye azophloxine (**2**) undergoes a strong color change upon complexation to Cp*Rh. The UV/Vis absorption spectrum of **2** (25 μM) after the addition of increasing amounts of **1** in aqueous phosphate buffer solution (100 mM, pH 7.0) is shown in Figure 1.

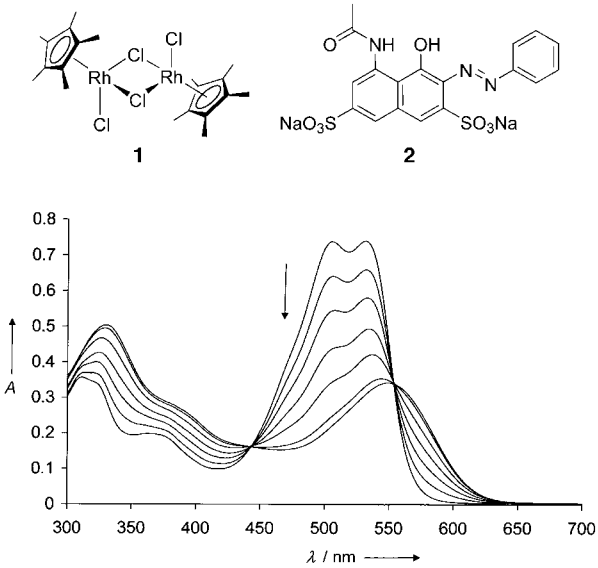

Figure 1. UV/Vis absorption spectra of a solution of **2** (25 μM) in aqueous phosphate buffer solution (100 mM, pH 7.0) upon addition of complex **1** (final Rh concentration: 0, 4.8, 9.6, 14.4, 19.2, 24.0 and 26.4 μM). The spectra were recorded after equilibration.

The complexation of the indicator to the rhodium complex results in a pronounced decrease in the absorption in the region of 500 nm with a new local maximum observed at $\lambda = 549$ nm. Clear isosbestic points are observed at $\lambda = 554$ and 443 nm which indicate that a single species is formed. It appears likely that the indicator is attached to the metal through the diazo and the phenolate groups as Cp*Rh complexes are known to form stable N,O-chelates with azophenolates.[14] At room temperature, the reaction between **1** (12.5 μM) and **2** (25 μM) proceeds with a half-life of $t_{1/2} = 2.5$ min; at 50 °C, the reaction is complete within 5 min. The interaction between the indicator and the rhodium complex is very strong: fitting of the titration data to a 1:1 binding algorithm yielded a binding constant of $3.2(\pm 1.0) \times 10^7\ M^{-1}$.[15,16]

Having confirmed that the indicator **2** is able to bind to the Cp*Rh complex with a strong concomitant change in color, we investigated whether a mixture of **1** and **2** could be employed to detect histidine- or methionine-containing peptides. The coordination chemistry of organometallic half-sandwich complexes with amino acids and peptides is well established.[17] It has been demonstrated that peptides preferentially bind to Cp^*Rh^{III}, Cp^*Ir^{III}, and (arene)Ru^{II} fragments through their terminal amino groups and deprotonated amide bonds. For histidine and methionine, a further interaction between the N- or S-donor group of the sidechain and the metal is generally observed. To determine whether this latter interaction allows a differentiation between pep-

[*] A. Buryak, Prof. K. Severin
Institute of Chemical Sciences and Engineering
Swiss Federal Institute of Technology Lausanne (EPFL)
1015 Lausanne (Switzerland)
Fax: (+41) 21-693-9305
E-mail: kay.severin@epfl.ch

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tides, we first examined competition experiments with **1**, **2**, and the dipeptides His-Ala or Val-Phe. Upon introduction of one equivalent of His-Ala to a mixture of **1** and **2** ([Rh] = [**2**] = 50 μM) in buffered aqueous solution, the original red color of the free indicator **2** reappeared. On the other hand, no color change was observed if the indicator **2** was added to a solution of **1** and His-Ala. This showed that the stability of the complex between Cp*Rh and His-Ala is considerably higher than that of the adduct between **1** and **2**. With Val-Phe instead of His-Ala, the purple color of the adduct between the receptor **1** and the indicator **2** was observed (Figure 2).[18] The affinity of Val-Phe to the Cp*Rh complex is thus not sufficient to displace the indicator **2** to a significant extent.

Figure 2. "Naked-eye" detection of the dipeptide His-Ala by an indicator-displacement assay (IDA) by using a solution of receptor **1** (50 μM), the indicator **2** (100 μM), and the respective dipeptide (100 μM) in aqueous phosphate buffer solution (100 mM, pH 7.0).

A more detailed analysis of competition experiments was performed by using UV/Vis spectroscopy to determine the relative binding constants $K_r = K_{peptide}/K_{indicator}$ of the Cp*Rh complex **1** to various peptides.[19] A variable amount of the respective peptide (final concentration: 0–26 μM) was mixed with the receptor (final Rh concentration: 12 μM) and the indicator (final concentration: 50 μM). After equilibration (50 °C, 10 min), the amount of free indicator was determined by using a calibration curve and the resulting data were fitted to calculate K_r.[16,20] The results are summarized in Table 1.

Upon addition of peptides that contain either His or Met residues in positions one or two from the N terminus, near complete replacement of the dye was observed ($K_r \geq 740$, entries 1–6). This holds true even for peptides such as Leu-His-Leu, in which the His residue is flanked by amino acids with sterically demanding side chains (entry 4). It is reasonable to assume that for peptides with His/Met residues at (or directly adjacent to) the N terminus, a simultaneous coordination of the amino acid and the respective sidechain occurs.[21] For tri- or longer peptides with His/Met residues in the middle or at the C terminus, this is less favorable and consequently lower relative binding constants are observed (entries 7–10). From the values observed for Gly-Gly-His and Gly-Gly-Met (entries 7 and 10), the interaction of the imidazole sidechain of histidine with the Cp*Rh complex seems to be stronger than the interaction of the thioether sidechain of methionine.[22] Five peptides without His/Met residues were also investigated (including dipeptides with basic and acidic sidechains),[23] and they all showed a very low relative binding constant. Remarkably, the substitution of a single His residue with an Ala residue results in a drop in affinity of more than five orders of magnitude (entries 1 and 12).

The high selectivity of the Cp*Rh receptor allows IDAs to be performed for the quantitative detection of peptides that contain His/Met residues close to the N terminus. For the detection of His-Ala, for example, the receptor **1** (final Rh concentration: 12 μM) and the indicator **2** (final concentration: 50 μM) were added successively to a solution that contained a variable amount of peptide (0–10 μM).[24] The resulting mixture was tempered for 10 min at 50 °C, and then the absorption at 580 nm was determined.[25] The corresponding calibration curve is depicted in Figure 3. The detection limit of this assay was found to be 0.3 μM.[26] In similar experiments with Val-Phe in place of His-Ala, no significant change in absorption was observed. This pronounced selectivity allows (low) micromolar concentrations of His-Ala to be detected in the presence of a 100-fold excess of Val-Phe (Figure 3).

A unique advantage of IDAs is that the indicator–receptor ratio can be adjusted for specific sensing problems.[9] It is thus

Table 1: Relative binding constants $K_r = K_{peptide}/K_{indicator}$ of the Cp*Rh complex **1** to various peptides.

Entry	Peptide	K_r[a]
1	His-Ala	$>1\times10^3$
2	His-Gly-Gly	$>1\times10^3$
3	Gly-His-Gly	$>1\times10^3$
4	Leu-His-Leu	$>1\times10^3$
5	Gly-Met-Gly	$>1\times10^3$
6	Met-Leu-Phe	$7.4\times10^2(\pm1.5\times10^2)$
7	Gly-Gly-His	$1.6\times10^1(\pm0.2\times10^1)$
8	Tyr-Gly-Gly-Phe-Met-Arg-Phe	$6.9(\pm0.2)$
9	Ala-Ser-His-Leu-Gly-Leu-Ala-Arg	$9.1\times10^{-1}(\pm0.2\times10^{-1})$
10	Gly-Gly-Met	$1.7\times10^{-1}(\pm0.2\times10^{-1})$
11	Val-Gly-Gly	$7.4\times10^{-2}(\pm0.2\times10^{-2})$
12	Ala-Ala	$2.2\times10^{-3}(\pm0.3\times10^{-3})$
13	Pro-Glu	$<1\times10^{-3}$
14	Val-Phe	$<1\times10^{-4}$
15	Lys-Tyr	$<1\times10^{-4}$

[a] Conditions: phosphate buffer (100 mM), pH 7.0.

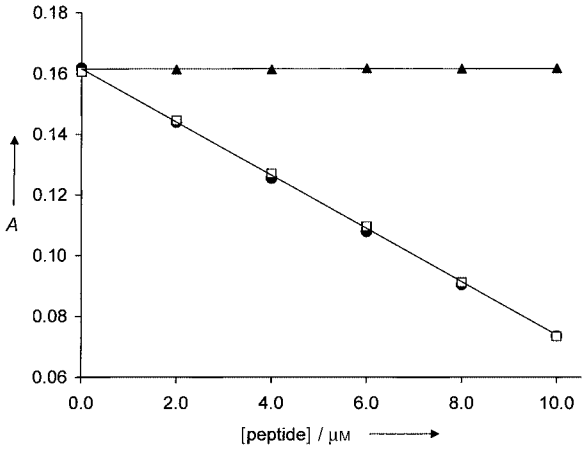

Figure 3. Absorbance at 580 nm for solutions containing receptor **1** (6 μM), the indicator **2** (50 μM), and different amounts of His-Ala (●), Val-Phe (▲), or His-Ala in the presence of a 100-fold excess of Val-Phe (□). The data points represent the average values of three independent experiments; the errors are less than 2%.

possible to quantify *and* distinguish the tripeptides His-Gly-Gly and Gly-Gly-His in two sets of experiments. Assuming that the peptides are present in concentrations between 0 and 10 μM, one would first have to perform an IDA with excesses of receptor and indicator ([Rh] = 50 μM; [**2**] = 60 μM). Under these conditions, the near-quantitative displacement of the indicator by both peptides is observed which allows their concentration to be determined without knowing their identity. In a second experiment, the concentration of the receptor is decreased to the value determined in the first experiment. Because of the excess of the indicator, only the high affinity analyte His-Gly-Gly completely displaces the indicator, whereas for the lower affinity analyte Gly-Gly-His, the UV/Vis response is much smaller. On the basis of these data, it is possible to identify the two peptides. The experimental values for peptide concentrations of 5 μM are given in Table 2.

Table 2: Differences in the absorbance at 504 nm for solutions of the dipeptides His-Gly-Gly and Gly-Gly-His after addition of various amounts of receptor **1** and indicator **2**.

Peptide	[Peptide][a]	[**1**][a]	[**2**][a]	ΔA
His-Gly-Gly	5.0 μM	25 μM	60 μM	0.108
Gly-Gly-His	5.0 μM	25 μM	60 μM	0.103
His-Gly-Gly	5.0 μM	2.5 μM	60 μM	0.108
Gly-Gly-His	5.0 μM	2.5 μM	60 μM	0.057

[a] Final concentrations are given.

In summary, we have shown that an organometallic 4d transition-metal complex can be used to build an IDA for the sequence-specific detection of histidine- and methionine-containing peptides in water at neutral pH. Although the assay is not suited for real-time measurements, it can be completed within a few minutes. The following characteristics make this new system especially appealing: a) the receptor **1** and the indicator **2** are both commercially available; b) the interaction of the receptor with the analyte is very strong which allows peptides with His/Met residues close to the N terminus to be detected at concentrations as low as 0.3 μM; c) the receptor displays a very high selectivity, which allows the analysis to be performed in the presence of phosphate buffer (100 mM) and in the presence of a large excess of competing peptides.

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[1] For reviews, see: a) S. L. Wiskur, H. Aït-Haddou, J. J. Lavigne, E. V. Anslyn, *Acc. Chem. Res.* **2001**, *34*, 963 – 972; b) L. Fabbrizzi, M. Licchelli, A. Taglietti, *Dalton Trans.* **2003**, 3471 – 3479; c) C. Sukasai, T. Tuntulani, *Chem. Soc. Rev.* **2003**, *32*, 192 – 202.

[2] a) S. C. McCleskey, A. Metzger, C. S. Simmons, E. V. Anslyn, *Tetrahedron* **2002**, *58*, 621 – 628; b) A. Metzger, E. V. Anslyn, *Angew. Chem.* **1998**, *110*, 682 – 684; *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 649 – 652.

[3] J. J. Lavigne, E. V. Anslyn, *Angew. Chem.* **1999**, *111*, 3903 – 3906; *Angew. Chem. Int. Ed.* **1999**, *38*, 3666 – 3669.

[4] S. L. Wiskur, E. V. Anslyn, *J. Am. Chem. Soc.* **2001**, *123*, 10109 – 10110.

[5] Z. Zhong, E. V. Anslyn, *J. Am. Chem. Soc.* **2002**, *124*, 9014 – 9015.

[6] a) S. L. Tobey, E. V. Anslyn, *Org. Lett.* **2003**, *5*, 2029 – 2031; b) Z. Zhong, E. V. Anslyn, *Angew. Chem.* **2003**, *115*, 3113 – 3116; *Angew. Chem. Int. Ed.* **2003**, *42*, 3005 – 3008; c) M. S. Han, D. H. Kim, *Angew. Chem.* **2002**, *114*, 3963 – 3965; *Angew. Chem. Int. Ed.* **2002**, *41*, 3809 – 3811; d) L. Fabbrizzi, N. Marcotte, F. Stomeo, A. Taglietti, *Angew. Chem.* **2002**, *114*, 2665 – 2668; *Angew. Chem. Int. Ed.* **2002**, *41*, 3811 – 3814; e) K. Niikura, A. Metzger, E. V. Anslyn, *J. Am. Chem. Soc.* **1998**, *120*, 8533 – 8534.

[7] L. Fabbrizzi, A. Leone, A. Taglietti, *Angew. Chem.* **2001**, *113*, 3156 – 3159; *Angew. Chem. Int. Ed.* **2001**, *40*, 3066 – 3069.

[8] a) M. A. Hortalá, L. Fabbrizzi, N. Marcotte, F. Stomeo, A. Taglietti, *J. Am. Chem. Soc.* **2003**, *125*, 20 – 21; b) K. E. S. Deam, G. Klein, O. Renaudet, J.-L. Reymond, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1653 – 1656; c) H. Aït-Haddou, S. L. Wiskur, V. M. Lynch, E. V. Anslyn, *J. Am. Chem. Soc.* **2001**, *123*, 11296 – 11297; d) G. Klein, J.-L. Reymond, *Angew. Chem.* **2001**, *113*, 1821 – 1823; *Angew. Chem. Int. Ed.* **2001**, *40*, 1771 – 1773.

[9] A. M. Piątek, Y. J. Bomble, S. L. Wiskur, E. V. Anslyn, *J. Am. Chem. Soc.* **2004**, *126*, 6072 – 6077.

[10] For details about an IDA for nitric oxide which is based on a rhodium complex, see: S. A. Hilderbrand, M. H. Lim, S. J. Lippard, *J. Am. Chem. Soc.* **2004**, *126*, 4972 – 4978.

[11] a) Z. Grote, M.-L. Lehaire, R. Scopelitti, K. Severin, *J. Am. Chem. Soc.* **2003**, *125*, 13638 – 13639; b) M.-L. Lehaire, R. Scopelitti, H. Piotrowski, K. Severin, *Angew. Chem.* **2002**, *114*, 1477 – 1480; *Angew. Chem. Int. Ed.* **2002**, *41*, 1419 – 1421; c) H. Piotrowski, K. Severin, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 4997 – 5000; d) H. Piotrowski, G. Hilt, A. Schulz, P. Mayer, K. Polborn, K. Severin, *Chem. Eur. J.* **2001**, *7*, 3196 – 3208; e) H. Piotrowski, K. Polborn, G. Hilt, K. Severin, *J. Am. Chem. Soc.* **2001**, *123*, 2699 – 2700.

[12] K. Severin, *Coord. Chem. Rev.* **2003**, *245*, 3 – 10.

[13] F. A. Dunand, L. Helm, A. E. Merbach, *Adv. Inorg. Chem.* **2003**, *54*, 1 – 69.

[14] F. Hintermaier, F. Kühlwein, C. Robl, W. Beck, *Z. Anorg. Allg. Chem.* **1996**, *621*, 829 – 836.

[15] The titrations were performed with **2** (12.0 μM) and **1** (0–20 μM) in aqueous phosphate buffer solution (100 mM) at pH 7.0.

[16] The data were fitted with the help of the Gepasi program, version 3.30 using a preinstalled Levenberg–Marquardt algorithm; a) P. Mendes, *Comput. Appl. Biosci.* **1993**, *9*, 563 – 571; b) P. Mendes, *Trends Biochem. Sci.* **1997**, *22*, 361 – 363.

[17] K. Severin, R. Bergs, W. Beck, *Angew. Chem.* **1998**, *110*, 1722 – 1743; *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 1635 – 1654, and references therein.

[18] For the "naked-eye" detection of histidine-containing peptides, the required minimum concentration is approximately 20 μM.

[19] $K_{peptide}$ is defined as: $K_{peptide} = [Cp^*Rh—peptide\ complex]/[Cp^*Rh(L_n)][peptide]$ with L_n being aqua, chloro, and/or phosphate ligands. $K_{indicator}$ is defined accordingly.

[20] To estimate K_r for peptides without His/Met residues, a large excess of the respective peptide (final concentration: 300–500 μM) was mixed with the receptor (final concentration: 25 μM) and the indicator (final concentration: 25 μM).

[21] Besides the N-terminal amino group and the sidechain, an amide group can potentially participate in coordination.

[22] The thermodynamic preference for the coordination of N-donor ligands in competition to S-donor ligands has also been observed for a Zn^{II}-containing receptor and is in accordance with

theoretical studies of Pt^{II} complexes; a) A. T. Wright, E. V. Anslyn, *Org. Lett.* **2004**, *6*, 1341 – 1344; b) D. V. Deubel, *J. Am. Chem. Soc.* **2004**, *126*, 5999 – 6004.
[23] Redox-active Cys-containing peptides were not included in our study.
[24] Stock solutions were employed for the receptor and the indicator.
[25] The largest relative changes in absorption are observed at 599 nm but owing to reasons of sensitivity (larger absolute changes) it is advantageous to perform the assay at 504 nm or at ~580 nm (see Figure 1).
[26] The detection limit was assumed to be $3\,s_{blank}$ (s = standard deviation). The limit for the quantitative detection is approximately 1.0 μM ($10\,s_{blank}$); see: J. C. Miller, J. N. Miller, *Statistics for Analytical Chemistry*, Ellis Horwood Limited, Chichester, **1984**, pp. 96 – 100.

