Array-based sensing of purine derivatives with fluorescence dyes

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Paper

Array-based sensing of purine derivatives with fluorescence dyes

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Natural and synthetic purine derivatives such as caffeine, theophylline, 6-mercaptopurine and 8-chlorotheophylline are important drugs. Due to the structural similarity of these compounds, it is intrinsically difficult to prepare chemosensors for their selective optical detection. Here, we describe a sensor array which can be used to differentiate pharmacologically important purine derivatives with good accuracy. The array is composed of four polysufonated fluorescent dyes, all of which can bind purines via π-stacking interactions. The complexation of the analytes results in partial quenching of the fluorescence. The fluorescence response of the four dyes provides a characteristic signal pattern, enabling the identification of thirteen purine derivatives at low millimolar concentration. Furthermore, it is possible to use the array for obtaining information about the quantity and purity of purine samples.

Introduction

Naturally occurring purine alkaloids are pharmacologically important compounds. Caffeine is a stimulus of the central nervous system (CNS) and "the world's most popular drug".[1] The structurally related theophylline is a bronchodilator which is used by people suffering from respiratory problems such as asthma.[2] The isomers theobromine and paraxanthine are – like caffeine – stimuli of the CNS.[3] Some synthetic purine derivatives have also gained importance as drugs. 8-Chlorotheophylline, for example is given in combination with the antihistamine diphenhydramine as a drug against motion sickness.[4] The simple 6-mercaptopurine is an immunosuppressive drug which is used during the treatment of leukemia and inflammatory bowel disease, among others.[5] The N9-alkylated purine derivatives cladribine and penciclovir are important drugs as well, the former being used against hairy cell leukemia and multiple sclerosis,[6] whereas the latter is a key component for the treatment of herpesvirus.[7]

Given the biological and pharmacological importance of purine derivatives, it is not surprising that substantial efforts have been devoted to create synthetic receptors and optical sensors for these compounds.[8] Caffeine has been a particularly popular target, and numerous studies about the selective recognition[9] and/or sensing[10,11] of this drug have appeared over the last years. Other investigations have focused on the binding of natural methylxanthines (caffine, theophylline and theobromine) to RNA[12] or DNA,[13] on the complexation and the fluorescence sensing of theobromine via hydrogen bonds,[14,15] and on the binding of purine derivatives by molecularly imprinted polymers.[16]

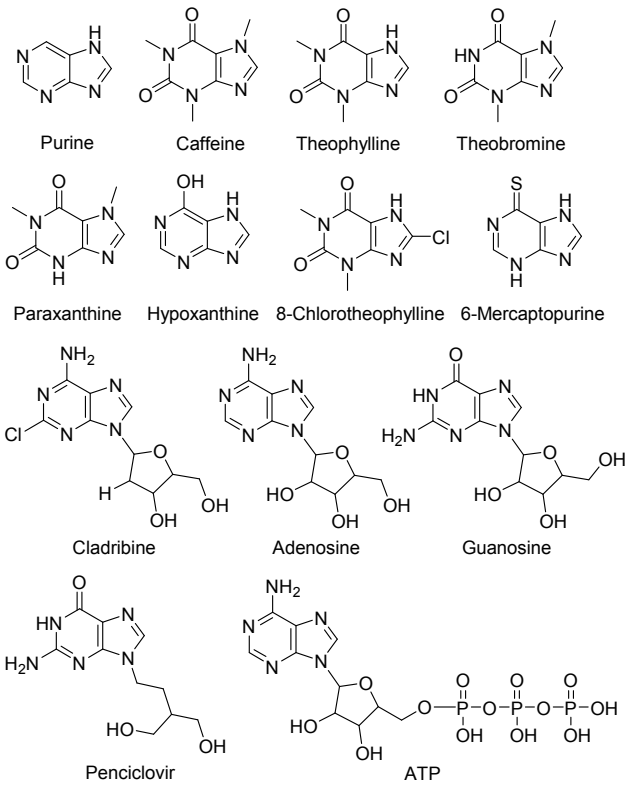

Fig. 1. Purine and some biological and pharmacological important derivatives.

A key difficulty in the preparation of selective receptors or chemosensors for purine derivatives is the structural similarity of these compounds. This is nicely illustrated by the published chemosensors for caffeine, which typically show a poor selectivity for caffeine over theophylline.[10,11] A sensing approach for which cross-reactivity is not an obstacle is the utilization of a sensor array. In a sensor array, the output of multiple sensors is combined to provide a characteristic signal pattern for an analyte or an analyte mixture.[17] The individual sensors do not have to be particularly selective, but they should display a different selectivity profile for the analytes of choice. Below, we demonstrate that a small set of polysulfonated fluorescent dyes can be used to create a powerful sensor array for purine derivatives. The array allows the identification of a series of important purines with good precision in the low millimolar concentration range. Furthermore, we show that it is possible to differentiate samples containing different amounts of caffeine or theophylline and samples containing mixtures of these compounds.

Results and Discussion

In 2011, we have reported that the commercially available fluorescent dyes trisodium 8-hydroxypyrene-1,3,6-trisulfonate (HPTS) and tetrasodium pyrene-1,3,6,8-tetrasulfonate (PTS) can be used as molecular probes for the detection of caffeine in water (Figure 2).[11b] The recognition of caffeine occurs via π-stacking interactions, which lead to partial quenching of the fluorescence. Both dyes display a moderate selectivity for caffeine over the dimethylxanthine derivatives theophylline and theobromine. In line with these results, we found that the apparent binding constant of HPTS for caffeine (K_a ~ 250 M^{-1}) is approximately twice as high as those for theophylline and theobromine. Guided by computational chemistry,[18] we have subsequently synthesized the fluorescent dye disodium 3,4:3',4'-bibenzo[*b*]thiophene-2,2'-disulfonate (TDS, Figure 2). This dye was found to display a unique fluorescence response upon addition of caffeine, which was attributed to non-covalent interactions of caffeine with the dye in the ground state and in the excited state.[11a] As a consequence, it was possible to use the dye for ratiometric measurements, thereby increasing the selectivity for caffeine over theophylline.

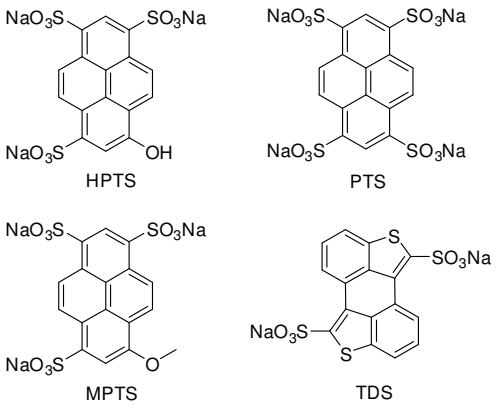

Fig. 2. The polysufonated fluorescent dyes used in this study.

Following these first investigations, we have examined the optical response of trisodium 8-methoxypyrene-1,3,6-trisulfonate (MPTS) upon addition of caffeine and theophylline (20 mM MOPS buffer, pH 7.0). These investigations were performed merely as control experiments, because we expected very similar results as observed for the structurally related dyes HPTS and PTS. Surprisingly, however, the selectivity profile of MPTS was inversed: a stronger fluorescence quenching was observed for theophylline than for caffeine (Figure 3). These results were corroborated by NMR titration experiments in D_2O, which showed that MPTS binds theophylline stronger than caffeine (see ESI, Figure S1 and S2).

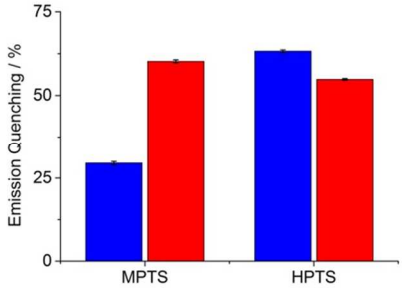

Fig. 3. Fluorescence emission quenching of buffered aqueous solutions (20 mM MOPS, pH 7.0) containing MPTS (λ_{ex}: 350 nm, λ_{em}: 430 nm) or HPTS (λ_{ex}: 460 nm, λ_{em}: 511 nm) upon addition of caffeine (blue) or theophylline (red) ($[dye]_{final}$ = 2.0 μM, $[analyte]_{final}$ = 5.0 mM). The values represent averages of 3 independent measurements.

The different selectivity of MPTS when compared to HPTS or PTS, and the unique optical response of TDS suggested to us that it might be possible to use a sensor array approach for the differentiation of structurally related purine derivatives. To test this hypothesis, we analyzed the thirteen purine derivatives shown in Figure 1 using the dyes HPTS, PTS, MPTS and TDS (Figure 2). This small array of four fluorescent dyes was used to generate a five-dimensional signal output, with TDS fluorescence being recorded at two different wavelengths (424 and 546 nm). The fluorescence measurements were performed in buffered aqueous solution (20 mM MOPS, pH 7.0) with a dye concentration of 2.0 μM and an analyte concentration of 1.0 mM. For each analyte, five independent measurements were performed.

A characteristic signal pattern was obtained for each of the ten analytes (Figure 4). As expected, the signal pattern is rather similar for the isomers theophylline, theobromine and paraxanthine, but on close inspection one can clearly detect differences in relative and absolute signal intensity. However, other analytes clearly stand out. 8-Chlorotheophylline, for example, leads to a rather selective quenching of HPTS fluorescence, and 6-mercaptopurine is an efficient and selective quencher for MPTS. For all dye/analyte combinations, we assume that fluorescence quenching is due to the formation of a π-stacked complex, an assumption which is in line with our previous computational studies.[11a,18] A significant contribution of other interactions such as hydrogen bonding seems unlikely, given that we use buffered water as the solvent. The characteristic fluorescence response of the individual dye/analyte pairs is a reflection of the different association constants, and of the different electronic properties of the dyes and the analytes.

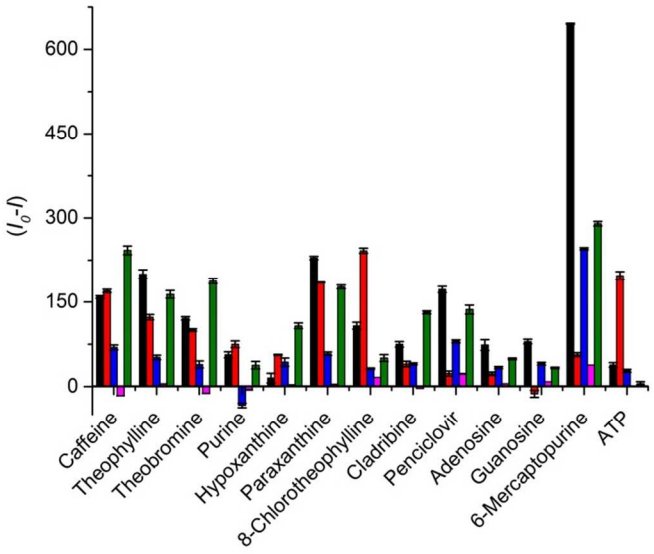

Fig. 4. Changes of the fluorescence emission intensities of buffered aqueous solutions (20 mM MOPS, pH 7.0) containing the dyes MPTS (λ_{ex}: 350 nm, λ_{em}: 430 nm, black), HPTS (λ_{ex}: 460 nm, λ_{em}: 511 nm, red), TDS (λ_{ex}: 350 nm, λ_{em}: 424 nm, blue; λ_{em}: 546 nm, pink) or PTS (λ_{ex}: 364 nm, λ_{em}: 384 nm, green) upon addition of caffeine, theophylline, theobromine, purine, hypoxanthine, paraxanthine, 8-chlorotheophylline, 6-mercaptopurine, cladribine or penciclovir ($[dye]_{final}$ = 2.0 µM, $[analyte]_{final}$ = 1.0 mM). The values represent averages of five independent measurements.

The array data was then processed by using a principal component analysis (PCA).[19] The corresponding two-dimensional score plot shows well separated data clusters (Figure 5), confirming that the individual purine analytes can be identified correctly. It is worth to point out that the two main principal components combined account for only 82.8% of the variance of the system. Factor 3 contributes 14.6% and factor 4 still 1.3%. These values indicate a rather high dimensionality, even though we have only used a small array.

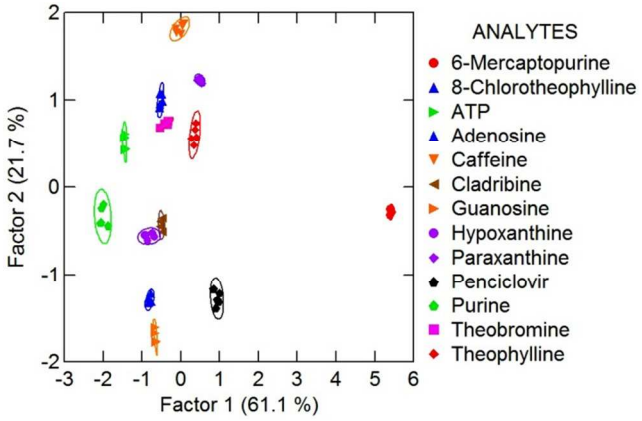

Fig. 5. Two-dimensional PCA score plot with confidence ellipsoids (p = 0.8) for the analysis of samples containing different purine derivatives ($[dye]_{final}$ = 2.0 µM, $[analyte]_{final}$ = 1.0 mM). The data were obtained as described in the main text.

To quantify the similarity of the signal patterns, we have used a hierarchical cluster analysis (HCA). The resulting HCA dendrogram confirms the similarity of the data from the isomers theophylline, theobromine and paraxanthine, and the unique position of the analyte 6-mercaptopurine.

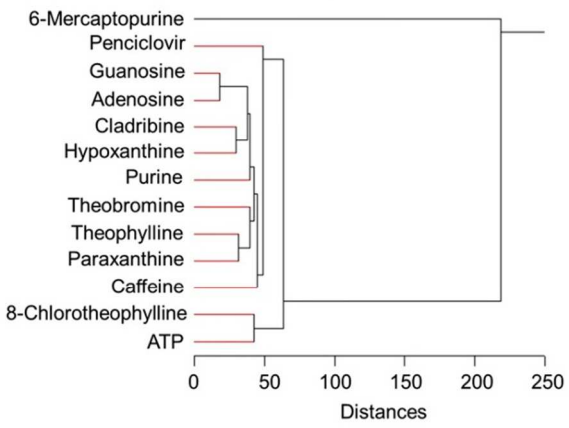

Fig. 6. HCA dendrogram derived from the array data.

To demonstrate the scope of the sensor array, we examined a more difficult analytical task: the differentiation of samples containing either caffeine or theophylline at different concentrations (1.0 – 5.0 mM). In addition, we have used five samples containing mixtures of caffeine and theophylline with a fixed total concentration of 5.0 mM. The entire set of 19 samples was then subjected to a sensor array analysis as described above. The PCA score plot indicates that it is possible to identify the samples containing pure caffeine or theophylline at a given concentration *and* to distinguish the samples which contain different caffeine-theophylline ratios (Figure 7).

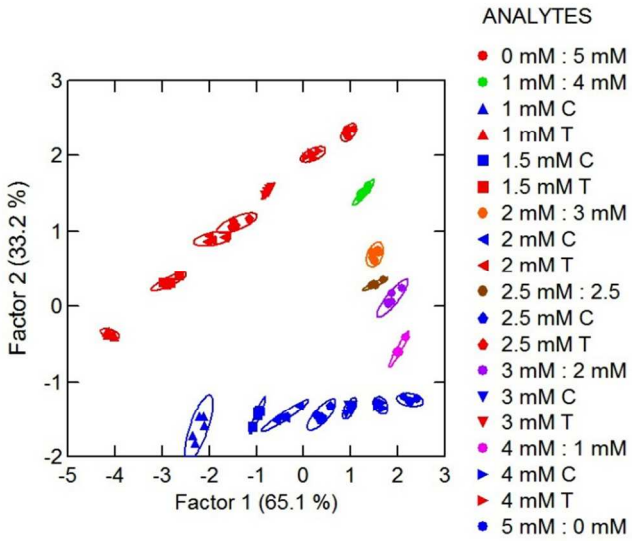

Fig. 7. Two-dimensional PCA score plot with confidence ellipsoids (p = 0.8) for the analysis of samples containing caffeine and/or theophylline at different concentrations and ratios ([dye] = 2.0 µM, [caffeine] = 1.0 – 5.0 mM; [theophylline] = 1.0 – 5.0 mM; [caffeine:theophylline mix] = 5.0:0 mM – 0:5.0 mM). The data were obtained as described in the main text.

In addition to the PCA, we have used the same data set to perform a linear discriminant analysis (LDA). A "Jack-knifed

matrix" validation procedure was then performed, randomly omitting one measurement at a time. The remaining data were used as the training set for the LDA, and the omitted observation was classified. This procedure resulted in a correct classification in 98% of all cases. For a real application (e.g. simultaneous quantification of caffeine and theophylline in a sample of unknown composition), it would be necessary to record a more comprehensive data set using additional test samples. The data could then be used to train an artificial neural network. The trained network would be able to make a prediction regarding the composition of unknown samples.[20]

In order to estimate the limit of detection for a quantitative analysis, we have examined the response of the four dyes with theophylline samples of 0.50 and 0.25 mM. A good signal-to-noise ratio was observed for 0.50 mM, but the 0.25 mM sample provided a too weak response with the dyes HPTS and TDS. It is thus recommended to use sample concentration of 0.50 mM or higher.

Conclusions

A small sensor array composed of four polysulfonated fluorescence dyes was used for the optical analysis of purine derivatives. The discriminatory power of the system is remarkably high, allowing the differentiation of thirteen different purines at a concentration of 1.0 mM with good precision. Furthermore, we have shown that it is possible to obtain information about the quantity *and* the purity of samples. This point was demonstrated by the analysis of samples containing different amounts of caffeine and/or theophylline. In terms of potential applications, one should note that an array approach with cross-reactive receptors is prone to interference from a complex matrix.[17,19] However, it could be a well suited method to achieve a fast and cheap quality control of synthetic samples. From a more fundamental point of view, it is noteworthy that an intrinsically non-specific interaction such as π-stacking can be used to differentiate structurally closely related compounds.

Experimental section

General: All chemicals and solvents were purchased from standard suppliers and used without further purification. Stock solutions were prepared with bi-distilled water and were stored at 4 °C. MOPS buffer (20 mM MOPS buffer, pH 7.0) was prepared by dissolving 3-(N-morpholino) propanesulfonic acid in bidistilled water. HCl and NaOH solutions were used to adjust the pH of the buffer. The dyes MPTS, HPTS, and PTS were obtained from commercial sources, and TDS was synthesized as described earlier.[10a]

Fluorescence measurements: Fluorescence measurements were performed on a Varian Cary Eclipse fluorescence spectrophotometer at room temperature. Stock solutions of the dyes (0.20 mM) and the purine analytes (1.0 – 5.0 mM) were prepared in MOPS buffer. For the measurements, aliquots of the respective stock solutions were mixed with MOPS buffer in quartz cuvettes to give a total volume of 1.5 mL. The final dye concentration was 2.0 μM; the concentration of the analytes in the different experiments is specified in the main text. The fluorescence of the samples was recorded 5 min after sample preparation.

Multivariate analysis: Five independent fluorescence measurements were performed for each sample with four different dyes. The following excitation and emission wavelengths were employed: MPTS: λ_{ex} = 350 nm, λ_{em} = 430 nm, HPTS: λ_{ex} = 460 nm, λ_{em} = 511 nm, TDS: λ_{ex} = 350 nm, λ_{em} = 424 nm *and* 546 nm, PTS: λ_{ex} = 364 nm, λ_{em} = 384 nm. The emission data were analyzed with the help of the statistics software SYSTAT 11. Entire fluorescence spectra for the different dye-analyte combinations are shown in the ESI.

Acknowledgements

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Notes and references

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Electronic Supplementary Information (ESI) available: experimental details and analytical data. See DOI: 10.1039/b000000x/

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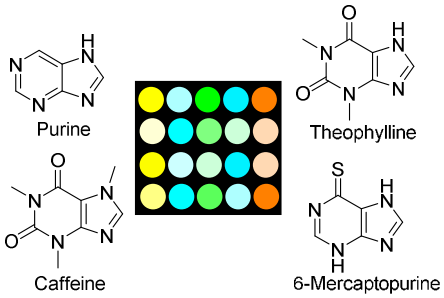
Graphic for the TOC



Text for the TOC: A sensor array based on polysulfonated fluorescent dyes can be used to differentiate purine and its derivatives with good precision.