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Supramolecular Approach for Sensing Caffeine by Fluorescence

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The host–guest interaction of receptors based on triphenylene ketals with oxopurines influences their fluorescence properties and can be exploited for sensing caffeine; this gives a rapid and easy insight into the binding behavior of synthetic caffeine receptors and their guests. This approach also permits a first insight into a methanol-induced self-aggregation of the receptors.

INTRODUCTION

Caffeine (**3**) is the most widely consumed alkaloid. The major natural sources are coffee, black tea and cocoa beans [1]. Caffeine is also found in many soft drinks and in various analgesics. Due to its adverse impact on minors and pregnant women, it is highly desirable to develop fast, convenient and reliable methods for the determination of the caffeine content in such formulations.

The standard methodologies for the quantitative analysis of caffeine involve chromatographic techniques such as (HP)TLC, HPLC-UV and CE [2]. Method development has shortened measurement times to a few minutes, but still a real on-line detection is impossible. This area is left to chemosensors [3], which represent a vivid field in supramolecular chemistry [4–10]. In line with these developments, several synthetic receptors for caffeine were developed in recent years [11–14], but for application as chemosensors most of them lack analytically useful responses to the binding event [15]. Due to the ease of application and measurement, spectrometric applications have attracted considerably the attention of many researchers in the field of chemical sensoring. Fluorimetric methods convince by their extreme sensitivity and the ease of designing the appropriate system [8,9]. Several sensoring principles require significant changes of the electronic structure and thus are limited to ionic analytes. In the recognition of neutral molecules, the boronic acid based sensors for diols as envisioned by Shinkai and Czarnik were ground breaking and set a start for a huge variety of developments [10,16–20]. Fluorescent chemosensors that rely exclusively on hydrogen bonding are reported as well [21–29].

As receptor we employed affinity systems (**1**, **2**) comprising a triphenylene-based ketal. A class of molecules based on this scaffold was developed in our group and the caffeine complexes of various receptors have been thoroughly analyzed by X-ray crystallography, NMR and CD spectroscopy [11,30–32]. The binding constants for the different systems in CH_2Cl_2 have been determined to be in the range of $40.000\,M^{-1}$.

The guest molecules involved in this study comprise caffeine (**3**), 7-octyltheophylline (**4**) and theophylline (**5**). Compound **4** was obtained following a procedure described by Bram et al. [33]. All three compounds reveal the same arrangement of hydrogen bonding acceptors that is complementary to that of the binding site. Some experiments were also performed with 1,3,7-trimethyluric acid (**6**), an oxidation product from caffeine and a common metabolite of oxopurines.

Trimethylxanthines like caffeine or 7-octyltheophylline (**4**) do not absorb light at wavelengths greater than 310 nm, whereas the triphenylene ketal still exhibits a significant absorption in the range up

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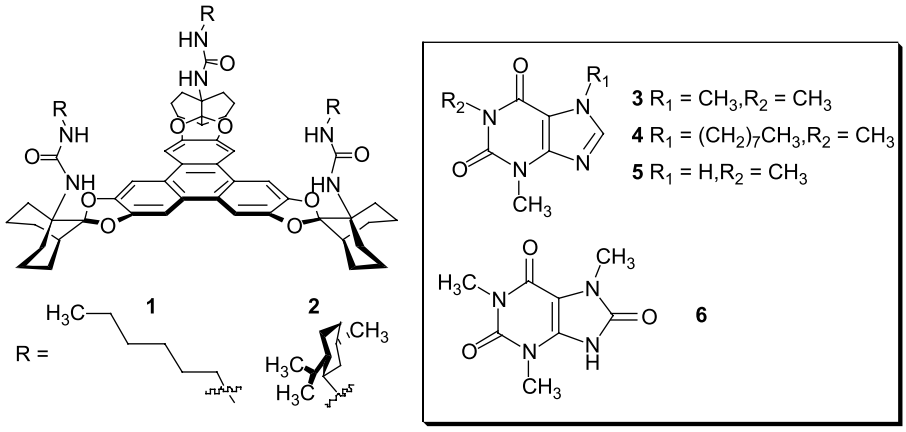

FIGURE 1 Receptor and guest molecules.

to 360 nm ($\varepsilon_{352} = 15.000\,M^{-1}\,cm^{-1}$, see Fig. 2). The low energy transitions (esp. $S_0 \rightarrow S_1$) are symmetry forbidden and thus low in intensity [34]. The emission occurs at 350–480 nm with a peak at 389 nm. Due to these properties, the excitation of the triphenylene moiety and subsequent fluorescence can occur without spectral interference of the guest. The excitation scan shows the same spectral structure as the UV. Furthermore, the emission spectra are insensitive to variation of the excitation energy.

Addition of caffeine to a solution of receptor results in a bathochromic shift of the emission bands (Fig. 2) by 2 nm and an increased signal intensity, especially at 388–393 nm. In the UV spectrum a bathochromic shift is observed for the lower excitations (S_1: 1.8 nm) as well. For hexaalkoxy triphenylenes a comparable, but more pronounced shift was found when going from solution to columnar mesophases [34,35]. The Lehn group described an increased fluorescence due to π-stacking of an adenosine group and an acridinium moiety in a chemosensor for ATP [36]. In the latter report a photoinduced electron transfer (PET) was identified as a reason for the finding. Here we expect that perturbations due to the binding of the guest make the symmetry-forbidden transition more feasible [37,38].

The signal change occurs instantaneously, i.e. in the setup with a stirring bar in the cuvette, signal stability is achieved after a mixing time of 10 s. This is in agreement with NMR spectroscopic results that have shown a highly dynamic exchange of the guest.

The determination of association constants using this effect is superior to NMR and CD spectroscopic methods, since only very little amounts of the receptor (1×10^{-5} M solution) are required for determination. In addition, the measurement is quick, robust and requires less expensive instrumentation.

The titration spectra for caffeine (**3**) and 7-octyltheophylline (**4**) reveal several isosbestic points, indicating the presence of two fluorescent species. The affinity constants for the caffeine complexes are comparable to those obtained by other methods (NMR, CD) [39]. Interestingly, the binding constant of 7-octyltheophylline (**4**) is in the

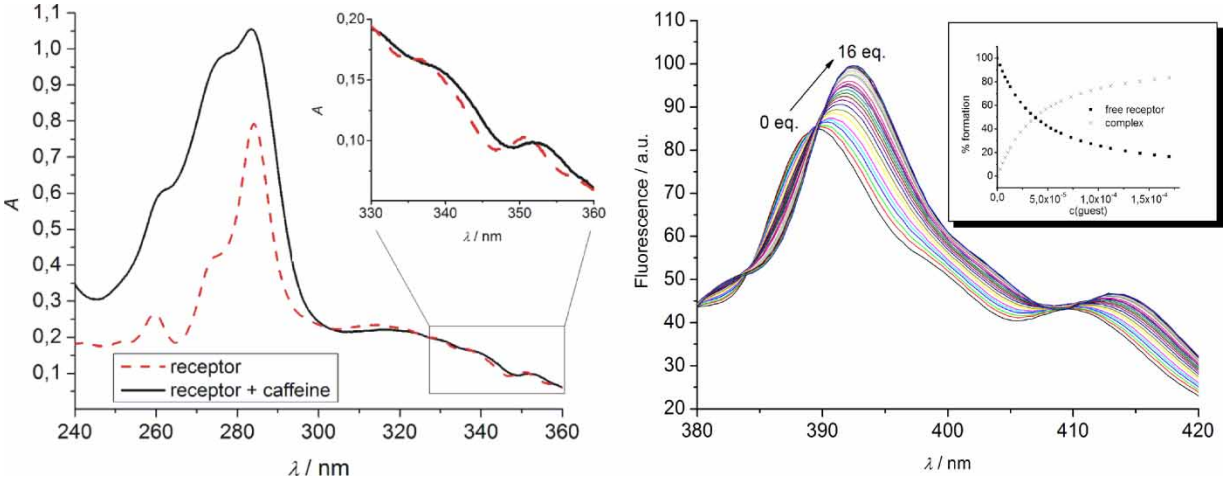

FIGURE 2 Spectroscopic properties of receptor **1** and its complexes with **3**. Left: UV–VIS; right: fluorescence titration, inset: species distribution plot as obtained from binding analysis.

TABLE I Log K obtained by fluorescence spectroscopy

Guest	Receptor	
	1	**2**
3	4.45 ± 0.05	4.48 ± 0.02
4	4.49 ± 0.02	4.4 ± 0.1
5	4.66 ± 0.05	4.44 ± 0.03

Titration conditions: c(receptor) $= 1 \times 10^{-5}$ M; $T = 25$°C.

same range as that for caffeine, indicating that the extended alkyl substituent does not interfere with the binding (Table I).

Theophylline reveals a very limited solubility in chlorinated solvents, most likely due to formation of hydrogen bonded aggregates. Due to the high dilution, binding of theophylline (**5**) could be investigated for the first time by spectroscopic tools. The apparent binding constant is comparable to that of caffeine [40]. Theophylline stands out in terms of signal increase. It is only approx. 90% of the value found for the alkylated derivatives. In addition, the bathochromic shift of the band is slightly smaller. However, these differences are too low to differentiate caffeine and theophylline. In the analysis of coffee beverages or caffeinated soft drinks this is a minor issue, since the content of theophylline and theobromine (1,7-dimethylxanthine) is relatively low compared to caffeine [41].

Employing a 2.5×10^{-5} M solution of receptor **1**, a calibration curve was obtained for the addition of caffeine to the receptor. Standard deviations were smaller than 2% after correction for offsets in a concentration range for caffeine of 5×10^{-6}–5×10^{-5} M. In order to show the general applicability of the method for quantitative analysis, a commercial over-the-counter analgesic was investigated [42]. Recovery rates in three subsequent measurements were 98–109%.

For application in natural matrices, it is necessary to cope with the absorption of either the exciting or the emitted light. As an alternative to the standard addition method it would be desirable to destroy the complexes in presence of the full matrix and thus obtain directly the value for the desired blind. Methanol as competing solvent should interfere with the hydrogen-bonding and thus break the complexes. And indeed, in a mixture of CH_2Cl_2/MeOH (4:1), the addition of caffeine does not change the spectra at all.

However, during the investigations it was found that addition of methanol induces slow changes in the emission spectrum of the receptor. The fluorescence intensity of the receptor increases over a period of 20–30 min in the full range of the spectrum. The same effect occurs in presence of caffeine as well. Some experiments revealed induction periods of up to 20 min before the described behavior occurred. The amount of the increase is lower than that for caffeine addition but still significant. Since a chemical reaction with methanol could be excluded, aggregation is the most likely reason for these results (Fig. 3).

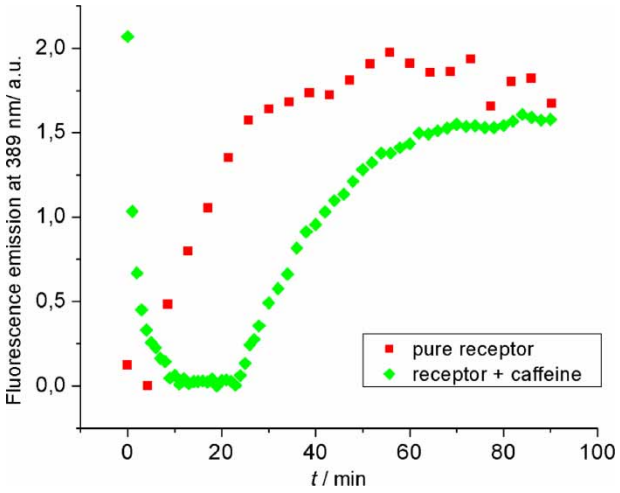

FIGURE 3 Representative fluorescence time traces of methanol induced aggregation. Prior to each experiment signal stability was checked by several measurements over periods of >10 min. Data shown for receptor **1** ($V = 2$ ml). $\lambda_{ex} = 352$ nm, $\lambda_{em} = 388.8$ nm. Addition of methanol ($V = 0.5$ ml) at $t = 0$ min.

This was confirmed by dynamic light scattering experiments (Table II). Assuming a spherical shape and standard density, the molecular mass of the particles can be estimated. Both receptors are found as single molecules if no methanol is present. Higher aggregates are not detected. Once the receptor solutions are spiked with methanol and allowed to stand for some time (>1 h) no more monomeric species can be found. In case of **1**, a dimer is the smallest aggregate found. The size of the higher aggregates could not be accurately observed due to their size dispersion and sedimentation.

By polarization experiments it was shown that the increase in fluorescence is not a result of scattering [43]. Methanol-mediated aggregation has been found for comparable systems and was investigated using X-ray crystallographic methods [44]. Apparently, the urea moieties play a crucial role in this network, while methanol acts as molecular "glue".

To our surprise, these systems are still able to bind a guest. 1,3,7-trimethyl uric acid binds in CH_2Cl_2 strongly to the receptor. Titration in CH_2Cl_2/MeOH

TABLE II Methanol-induced aggregation of receptors detected by dynamic light scattering; values given are for the smallest aggregate found; D_t = translational diffusion coefficient, R_h = hydrodynamic radius

Receptor	Solvent	D_t [cm^2s^{-1}]	R_h[nm]
1	CH_2Cl_2	3.78×10^{-6}	0.48
1	CH_2Cl_2/MeOH 4:1	2.85×10^{-6}	0.64
2	CH_2Cl_2	2.66×10^{-6}	0.7
2	CH_2Cl_2/MeOH 9:1	4.8×10^{-8}	38.6

(4:1) of receptor **1** with 1,3,7-trimethyl uric acid (1×10^{-3} M) gave the characteristic bathochromic shifts. Either the guest binds to the hosts located on the surface of the aggregate, or the binding is so advantageous, that the hosts return into solution. For further investigations, it would be necessary to repeat this experiment in pure dichloromethane. However, this is impossible due to the low solubility of 1,3,7-trimethyluric acid.

In conclusion, the fluorimetric detection of caffeine and related methylxanthines by use of a supramolecular receptor is possible. The changes observed are stronger than those observed for other known caffeine receptors. During the course of the investigations, it was found, that caffeine derivatives carrying extended alkyl substituents reveal a comparable affinity towards the receptor as the parent compound. Furthermore, this method revealed an unexpected, methanol induced self-aggregation, which could be exploited for the formation of controlled, surface-disposed layers. Investigations to increase the sensitivity of the systems are ongoing.

MATERIALS AND METHODS

All spectroscopic experiments were performed in analytical grade dichloromethane. All cuvettes were made of quartz glass, pathlengths/diameters of the cuvettes were in all cases 1 cm. UV–VIS measurements were performed on a Shimadzu 2010 UV–Vis double beam spectrophotometer. Fluorescence spectra were recorded on an Aminco-Bowman AB 2 spectrofluorometer. The excitation wavelength was 352 nm. Fluorescence experiments were conducted at a constant temperature of 25.00°C. Concentrations of receptor and guest for binding studies 1.0×10^5 M and $2.5–5.0 \times 10^{-3}$ M, respectively. The guest solution was added in portions of 1–10 μl to 2 ml of receptor solution. At least 15 data points were recorded in the range of 20–80% of complex formation. Binding constants were determined by global analysis of the evolution of the emission spectra by using Specfit V3.0. This software uses singular value decomposition and non-linear regression modeling by the Levenberg-Marquard- method [45]. In all models, only the existence of a 1:1 complex was assumed. Self-association of the receptor is negligible in absence of methanol and was thus omitted [11].

Dynamic light scattering experiments were performed with a DynaPro molecular sizing instrument (Protein Solutions) at a fixed angle of 90°. The Dynamics software package (Ver. 5.26.60) was used for data analysis. The translational diffusion coefficient (D) of the sample particles was determined by measuring the fluctuations in the intensity of scattered light with an autocorrelation function. The hydrodynamic radius of the particles was calculated using the Stokes-Einstein Eq. ($D_t = k_b T/6\pi\eta R$, where k_b is the Boltzmann constant, T is the absolute temperature and η is the solvent viscosity). Assuming that the particles are spherical and of standard density, the molecular mass of the particles was estimated from R_h.

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