Micelles Hot Paper

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Measurement of Local Sodium Ion Levels near Micelle Surfaces with Fluorescent Photoinduced-Electron-Transfer Sensors

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Abstract: *The Na^+ concentration near membranes controls our nerve signals aside from several other crucial bioprocesses. Fluorescent photoinduced electron transfer (PET) sensor molecules target Na^+ ions in nanospaces near micellar membranes with excellent selectivity against H^+. The Na^+ concentration near anionic micelles was found to be higher than that in bulk water by factors of up to 160. Sensor molecules that are not held tightly to the micelle surface only detected a Na^+ amplification factor of 8. These results were strengthened by the employment of control compounds whose PET processes are permanently "on" or "off".*

Biological membranes organize living matter in cells, create a fluid three-dimensional matrix, and allow for the controlled transport of solutes.[1] Many biologically important processes are membrane-mediated, yet surprisingly little is known about both reaction schemes and, more fundamentally, the complex nano-environments where reactions occur. For instance, the gradient of Na^+ concentration across biological membranes is involved in the transport of molecules into cells, pH homeostasis, and signal transmission in nerve systems,[2] and is regulated by proteins such as Na^+/K^+ ATPases,[3] Na^+/H^+ antiporters,[4] and voltage-gated Na^+ channels.[5] Accurate measurement of local Na^+ levels near model membranes, such as aqueous micelle surfaces,[6] requires sensors with excellent selectivity against ubiquitous H^+, but only very few are available.[7] Although the concentration of membrane-bounded H^+ has been determined with fluorescent sensors,[8] the concentrations of only a few larger ions (but not Na^+) have been measured near micelle surfaces and even then only with sensors whose fluorescence output is influenced by H^+.[9] The related field of ion-driven, micelle-bound logic systems has very few examples.[10] We have now determined local Na^+ levels near a micelle surface with designed photoinduced electron transfer (PET) sensors[11] for the first time.

The chemical structures of the fluorescent PET sensors (**1a**–**1d** and **4a**–**4b**) used in this study are shown in Figure 1. These sensors were designed based on the "fluorophore–spacer–receptor" concept.[12] The benzo-15-crown-5 structure was chosen as a receptor owing to its well-known binding affinity towards Na^+ (the $\log\beta_{Na^+}$ value of benzo-15-crown-5 is 0.4 in water).[13] Importantly, the Na^+ binding ability of the receptor is not affected by changes in the environmental pH value because it does not contain a pH-sensitive structure (e.g., an amino group). Anthracene was used as a fluorophore in **1a**–**1d** owing to its high hydrophobicity, which facilitates its uptake by micelles.[14] Furthermore, the anthracene structure

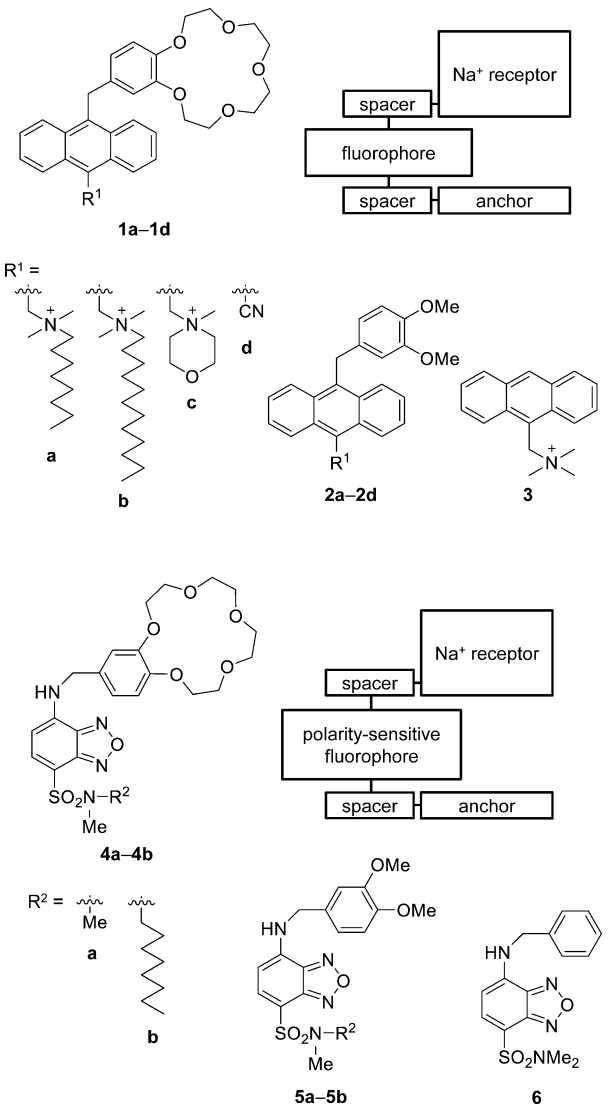

Figure 1. Chemical structures of the fluorescent Na^+ sensors (**1a**–**1d** and **4a**, **4b**) and control compounds (**2a**–**2d**, **3**, **5a**, **5b**, and **6**) used in this study. Counterions: Br^- for **1a**–**1c** and **2a**–**2c**, Cl^- for **3**.

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participates in a non-radiative PET process when R^1 is an electron-withdrawing substituent and the benzo-15-crown-5 unit is Na^+ free.[15] Otherwise, it emits strong fluorescence. Benzofurazan was also used as a fluorophore in some of the sensors (**4a**, **4b**). Along with the above characteristics described for anthracene, the benzofurazan structure has the remarkable property that the maximum emission wavelength is dramatically shifted to shorter values in a hydrophobic environment.[8c,16] In both cases (**1a**–**1d** and **4a**, **4b**), a short methylene spacer was used for efficient fluorescence switching based on the PET mechanism, and the anchor substituent was varied to change the local position of the sensor within the membrane-bound nanoenvironment.

2a–**2d** and **5a**–**5b** are critically important control compounds in studies of nanoenvironments by PET sensors as the dimethoxybenzene moiety is unable to bind Na^+ (i.e., the fluorescence is always "off"). Thus the fluorescence properties of **2a**–**2d** and **5a**–**5b** can only be altered by salt-induced environmental changes of the micelles (e.g., polarity changes). **3** and **6** are additional control compounds that never undergo PET (i.e., the fluorescence is always "on").[17]

In the present study, a variety of anionic, cationic, and neutral micelles were investigated as they all introduce different nanoenvironments. The chemical structures of the surfactants used in this study are shown in Figure 2. All of the

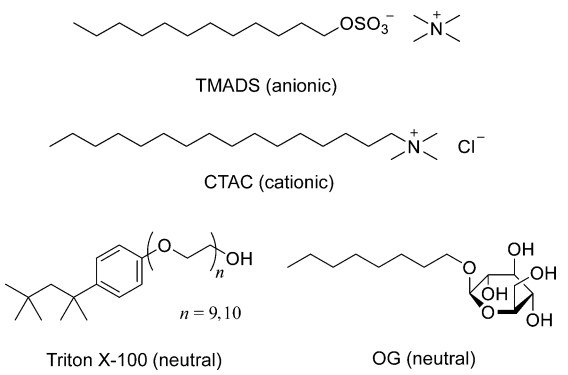

Figure 2. Chemical structures of the surfactants used in this study. Critical micelle concentration (cmc): 5.5 mM for tetramethylammonium dodecyl sulfate (TMADS),[22] 1.4 mM for cetyltrimethylammonium chloride (CTAC),[23] 0.24 mM for Triton X-100,[23] and 25 mM for octyl β-D-glucopyranoside (OG).[23]

micelles possess regions that are less polar than the surrounding aqueous environment but the presence of negatively charged, positively charged, and neutral head groups has a great influence over how the micelles interact with the surrounding environment. For instance, Na^+ ions are expected to be localized near the negatively charged head groups of the micelles because of electrostatic attraction.

The Na^+ concentration near micelle surfaces was evaluated by studying the fluorescence properties of the sensors as a function of bulk Na^+ concentration. This method, which had previously been applied to H^+,[8] was used for Na^+ sensing for the first time. The interaction between the fluorescent sensors (or control compounds) and the surfactants in micellar solutions could be confirmed by a dramatic increase in their solubilities compared to those in water. The hypsochromic shifts of the maximum emission wavelengths of the benzofurazan compounds (**4a**, **4b**, **5a**, and **5b**) in micellar solution (Table 1) also indicate that these sensors and control

Table 1: Maximum emission wavelengths of **4a**, **4b**, **5a**, and **5b**.

Solution	Wavelength[a] [nm] 4a	4b	5a	5b
TMADS (20 mM)	573	573	573	572
CTAC (5 mM)	573	574	573	573
Triton X-100 (0.52 mM)	546	541	563	558
OG (34 mM)	575	563	573	564
water	594	n.d.[b]	595	n.d.[b]

[a] Excited at the maximum absorption wavelength at 25 °C. [b] Could not be determined because of low solubility.

compounds are in a hydrophobic environment, that is, close to or inside the micelles. Representative fluorescence spectra of the sensors and control compounds in micellar solutions are shown in Figure 3 and 4. The most important result is that the

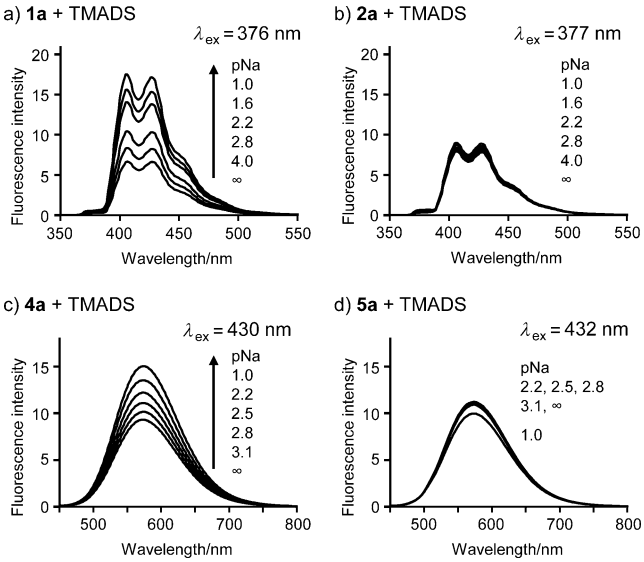

Figure 3. Representative fluorescence spectra with a variation in Na^+ concentration (pNa). a) **1a**, b) **2a**, c) **4a**, and d) **5a** (10 μM each) in TMADS solution (20 mM). pNa refers to the total concentration in the micellar solution and was varied by adding NaCl. The excitation wavelengths (λ_{ex}) are indicated in each panel.

fluorescence of the sensors is only switched on with increasing Na^+ concentration in TMADS solution (Figure 4; see also Figure S1 and Table 2). When the control compounds are used instead, such a behavior is not observed, indicating that the fluorescence of the sensors is switched on by Na^+ binding rather by a change in the micellar nanoenvironment (e.g., the local polarity) caused by salt effects.[18] However, the fluorescence of the sensors is not switched by a change in Na^+ concentration when they are placed in CTAC, Triton X-100, or OG solutions. Although it was reported that the binding

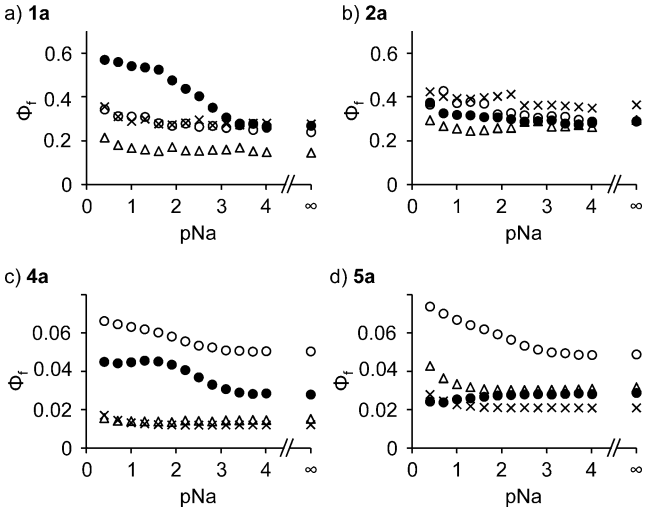

Figure 4. Dependence of the fluorescence quantum yield (Φ_f) on pNa for a) **1a**, b) **2a**, c) **4a**, and d) **5a** (10 μM) in TMADS (20 mM, ●), CTAC (5 mM, ○), Triton X-100 (0.52 mM, △), and OG (34 mM, ×) solutions. pNa refers to the total concentration in the micellar solution and was varied by adding NaCl.

Table 2: Fluorescence properties of the sensors and control compounds in TMADS solution.

Compound	$\Phi_{f,\,high\,[Na^+]}$[a]	$\Phi_{f,\,low\,[Na^+]}$[b]	FE[c]	$\log\beta_{Na^+}$
anthracenes				
1a	0.54	0.27	2.0	2.41 ± 0.18
2a	0.32	0.29		
1b	0.66	0.24	2.7	2.35 ± 0.02
2b	0.17	0.17		
1c	0.81	0.34	2.4	2.50 ± 0.04
2c	0.43	0.35		
1d	0.045	0.0056	8.1	1.26 ± 0.15
2d	0.010	0.0092		
3	0.81	0.78		
benzofurazans				
4a	0.045	0.028	1.6	2.57 ± 0.02
5a	0.025	0.029		
4b	0.057	0.038	1.5	2.56 ± 0.03
5b	0.031	0.039		
6	0.040	0.047		

[a] Na^+ concentration in solution: 0.1 M. [b] Na^+ concentration in solution: 0 M. [c] Fluorescence enhancement factor: $\Phi_{f,\,high\,[Na^+]}/\Phi_{f,\,low\,[Na^+]}$.

ability of benzo-15-crown-5 towards Na^+ increased in less polar media,[13] this effect was not observed even in the Triton X-100 micelles, which create the most hydrophobic environment for the sensors in the present study (see the maximum emission wavelengths in Table 1). This observation is due to the fact that ions avoid less polar membranes, preferring adjacent aqueous regions instead.[8c] Therefore, we conclude that Na^+ ions are mainly localized near TMADS micelles because of electrostatic attraction to the negatively charged sulfonate groups of the surfactant.

The local Na^+ concentration near TMADS micelles was measured by determining the $\Delta(\log\beta_{Na^+})$ value ($\log\beta_{Na^+}$ in TMADS solution $-\log\beta_{Na^+}$ in water) of the sensors,[19] analogous to the ΔpK_a method previously applied to micelle-bound H^+.[8a,20] The $\log\beta_{Na^+}$ values of the sensors in micellar solution were obtained using the equation:

$$\log[(I_{max}-I)/(I-I_{min})] = pNa-\log\beta_{Na^+}$$

where I, I_{max}, and I_{min} are the observed fluorescence intensity at a fixed wavelength and the corresponding maximum and minimum wavelengths, respectively (Table 2). Given the modular behavior of fluorescent PET sensors,[12] the $\log\beta_{Na^+}$ value in water is 0.4 (the value for benzo-15-crown-5).[13] On that basis, the $\Delta\log\beta_{Na^+}$ values were calculated to be 2.0 (for **1a** and **1b**), 2.1 (**1c**), 0.9 (**1d**), and 2.2 (**4a** and **4b**). Therefore, the local Na^+ concentration determined by the sensors near the TMADS micelle surface is 7.9–158 ($=10^{0.9}$–$10^{2.2}$) times higher than that in bulk water. The range of micelle-bound Na^+ concentrations found by these sensors can be ascribed to their different locations in the micellar system.[8c] Because of the Na^+ gradient created by the TMADS micelles, the neutral sensor **1d** would be located closer to the bulk water whereas the other cationic sensors are found closer to the micelle surface owing to ion paring with the head group. If the position of the sensors could be adjusted more extensively by structural variants of **1a**–**1d** and **4a**–**4b**,[8c] our method would allow for the determination of the Na^+ gradient near the micelle in more detail. The development of new receptors with stronger binding affinities towards Na^+ in aqueous media is also important because fluorescent sensors with such receptors will be able to determine the Na^+ concentration even near neutral and cationic micelles where Na^+ is repelled in comparison to bulk water because of dielectric effects and/or an electrostatic repulsion.

In conclusion, a series of new fluorescent PET sensors have been used to measure local Na^+ concentrations that are electrostatically increased in nanospaces[21] near anionic micelles for the first time. Similar experiments in nanospaces near more biorelevant membranes such as vesicles and liposomes are currently being conducted in our laboratories. Another important step for future biological use would be to improve the Na^+/K^+ selectivity of these sensors while preserving the pH independence. The diversity of available fluorescent PET sensor components for various targets[11,12] will also allow us to measure the concentrations of other important ions in biological nanoenvironments in a similar manner.

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