Analyte-induced aggregation of conjugated polyelectrolytes: role of the charged moieties and its sensing application†

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The vital role of the charged moieties of monovalent aromatic anions in regulating the aggregation of a cationic conjugated polyelectrolyte was exploited in terms of the hard–soft acid–base principle and its application to colorimetric sensing of taurine was examined.

Conjugated polyelectrolytes (CPEs) have attracted increasing interest during the last decade because of their potential for applications in sensing bioanalytes.[1–4] Hitherto, most CPE-based biosensors rely on a change in the emission intensity upon binding an oppositely charged analyte, and few CPEs can be applied to colorimetric detection.[2,3,5] Cationic poly(3-alkoxy-4-methylthiophene)s (P3RO-4MeT), a kind of water-soluble polythiophenes (PTs) with chain conformation sensitive to external stimuli, have been confirmed to be sensitive colorimetric probes for the detection of various bioanalytes.[3,6,7] The sensing mechanism for colorimetric detection has been attributed to the formation of CPE–analyte ion-pair complex, resulting in the conformation change and/or aggregation of PT backbones. For sensing small bioanions, analyte-induced aggregation of cationic P3RO-4MeT through electrostatic, hydrophobic and aromatic stacking cooperative interactions has been proposed to account for the remarkable solution color change.[7] There is also strong evidence that polyvalent bioanions with hydrophobic aromatic moieties facilitate the aggregation of cationic P3RO-4MeT and thus the colorimetric effect is further enhanced.[7] Although this sensing mechanism is based on specific lines of experimental evidence, some important issues remain unsolved.

As part of an ongoing investigation into the mechanism and application of aggregation-based CPE colorimetric sensors for small bioanions, we have an interest in examining the role of the charged moieties of aromatic anions in regulating aggregation of a cationic P3RO-4MeT derivative, poly(3-(4-methyl-3′-thienyloxy)propyltrimethylammonium) (PMTPA, Scheme 1a). It was confirmed that aromatic sulfonate can induce PMTPA aggregation much more efficiently relative to carboxylate and phosphate. This finding is applicable to detect sulfonate-containing bioanalyte, taurine, by combining with an *in situ* premodification technique. To the best of our knowledge, this is the first example of using a colorimetric CPE probe for detecting a monovalent bioanalyte in aqueous media.

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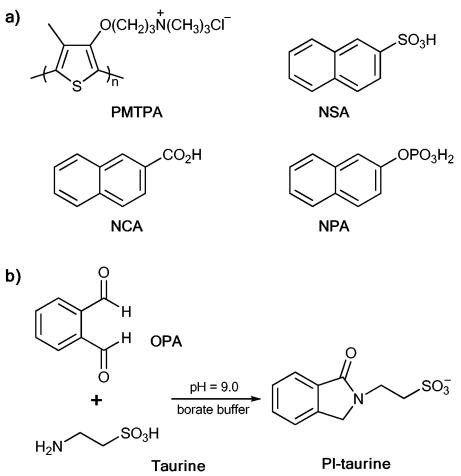
To investigate the influence of the charged moieties of the analytes on the aggregation of PMTPA, 2-naphthalenesulfonic acid (NSA), 2-naphthalenecarboxylic acid (NCA) and 2-naphthylphosphoric acid (NPA) (Scheme 1a) were chosen as the model analytes. Fig. 1 compares the absorption spectra of PMTPA in the presence of different amount of NSA, NCA and NPA. As shown in Fig. 1, the absorption maximum of PMTPA in 20 mM borate buffer (pH = 9.0) appears at 407 nm, being attributed to a random-coiled conformation of the PT backbone.[8] Upon adding increasing amounts of NSA, the absorption maximum is gradually red-shifted and finally featured with two major peaks at 543 and 589 nm, and a broad shoulder around 504 nm, being characteristic of the formation of a π-stacked supramolecular complex.[8b] Simultaneously, a distinct solution color change from yellow to purple was observed. In comparison, the introduction of NCA and NPA into aqueous PMTPA solution leads to different spectral features, in which the absorption maxima are blue-shifted to 376 and 380 nm, respectively, and the PMTPA solutions remain yellow. These observations indicated that NSA is a stronger aggregation-inducing molecule relative to NCA and NPA, which can be interpreted qualitatively by Pearson's hard–soft acid–base (HSAB) principle.[9] It is known that the quaternary ammonium group in PMTPA acts as a soft acid and sulfonate is a soft base, whereas both carboxylate and phosphate are hard bases. According to the HSAB principle, there is an extra stabilization in a hard–hard combination, or a soft–soft one. Therefore, one can conclude that the



Scheme 1 (a) Chemical structure of PMTPA, NSA, NCA and NPA. (b) Schematic illustration of the *in situ* premodification reaction of taurine with OPA.

electrostatic interaction between the quaternary ammonium group and sulfonate group is stronger than that with carboxylate or phosphate group.[10] Moreover, ionic self-assembly is characteristic of a cooperative binding mechanism.[11] Thus, the stronger primary electrostatic interaction would stimulate and enhance further secondary interactions including π–π stacking and hydrophobic interactions, facilitating the formation of π-stacked PMTPA aggregates.

On the basis of the new insights into the analyte-induced aggregation mechanism discussed above, colorimetric sensing of taurine (2-aminoethanesulfonic acid) based on the PMTPA probe was examined. Taurine, a sulfur-containing semi-essential amino acid, not only plays a key role in metabolism, but also can be a medicine to improve therapy of some diseases.[12] So far, several techniques have been devoted to detecting it, such as high-performance liquid chromatography (HPLC),[12] high-performance anion-exchange chromatography coupled with electroanalysis[13] and capillary electrophoresis with laser-induced fluorescence detection.[14] However, to a certain extent, there exist some limitations related to these methods. For example, they need relatively time-consuming procedures and expensive equipment. Therefore, it is still necessary to find new approaches that could improve the simplicity and selectivity of taurine detection.

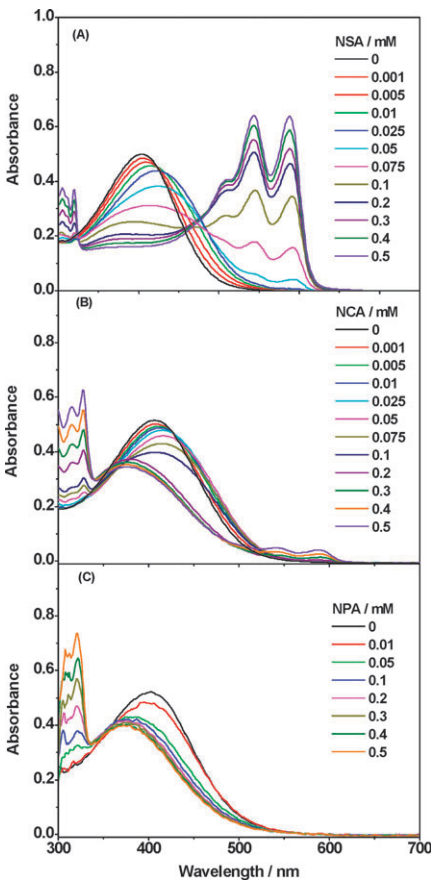


Fig. 1 Absorption spectra of PMTPA (1.0×10^{-4} M) in the presence of different amounts of NSA (A), NCA (B) and NPA (C) in 20 mM borate buffer (pH = 9.0). The bands between 300–350 nm are attributed to naphthalene moieties.

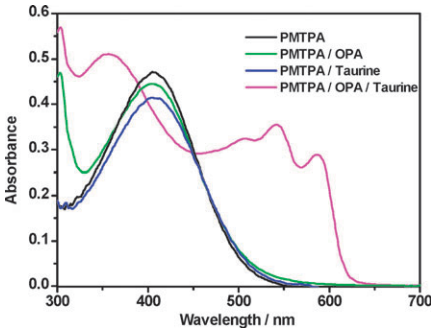


Fig. 2 Absorption spectra of PMTPA (1.0×10^{-4} M) in the absence and the presence of OPA, taurine and a OPA–taurine mixture in borate buffer (20 mM, pH = 9.0): [OPA] = 7.5×10^{-4} M; [taurine] = 5.0×10^{-4} M.

Taurine, having no aromatic moiety, interacts weakly with PMTPA in borate buffer, and scarcely induces any spectral change of PMTPA except for slightly weakening the absorption intensity (Fig. 2 and S1, ESI†). It is known that reaction of *o*-phthalaldehyde (OPA) with primary amine leads to the formation of the phthalimidine (PI) derivative.[15] Therefore, taurine can be converted into the sulfonate-containing PI derivative (PI-taurine) by the reaction with OPA (Scheme 1b).[16] The introduction of an aromatic group into taurine is expected to enhance interactions with PMTPA. Indeed, upon addition of OPA–taurine (a mixture of OPA and taurine pre-reacted for 3 min) into the PMTPA solution the absorption maximum was red-shifted to 541 nm along with the appearance of two broad shoulders around 507 and 587 nm (Fig. 2), being characteristic of the aggregation of PMTPA chains.[7] Simultaneously, a distinct color change from yellow to red–pink was observed, which was not achieved in the case of using OPA and taurine only. These results indicated that PMTPA could be a promising probe for the colorimetric sensing taurine by combining an *in situ* premodified approach.

To evaluate the specificity of PMTPA toward taurine, absorption spectra of PMTPA in borate buffer upon addition of OPA and sulfur-containing amino acids, methionine (Met), cysteine (Cys), homocysteine (Hcy) and cystine (Cyt), were recorded under identical conditions.[17] It was found that upon addition of these bioanions, all the solutions remained yellow with $\lambda_{max} < 430$ nm (see Fig. S3, ESI†). However, the most remarkable effect was observed for taurine, which gave a red–pink solution (Fig. 3). Thus, one would conclude that the dramatic color change of PMTPA upon addition of OPA–taurine provides a simple means for visual detection of taurine in aqueous solutions. The ratio of the absorbance difference between those at 600 nm (π-stacked aggregates) and 700 nm (light-scattering from the aggregates) to the absorbance at 407 nm (PMTPA random coil), $(A_{600} - A_{700})/A_{407}$, was calculated to further estimate the selectivity of PMTPA toward taurine (Fig. 4). It is clearly seen that the most striking effect is observed for taurine with the ratio value of 9.5× higher at least than that containing the other amino acids. These results indicate that PMTPA is selectively responsive to taurine and confirms that the charged moiety of the analyte plays a key role in controlling the ionic

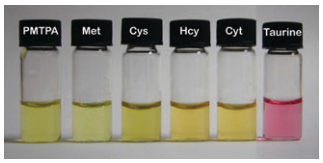


Fig. 3 Changes in the color of PMTPA solutions (1.0×10^{-4} M) in borate buffer (pH = 9.0) induced by addition of various OPA–amino acid mixtures: [OPA] = 7.5×10^{-4} M; [amino acid] = 5.0×10^{-4} M.

self-assembly of PMTPA to form π-stacked aggregates. To further verify this conclusion, PI-β-alanine (PI-β-Ala, see Fig. S4, ESI†) induced-aggregation ability towards PMTPA was examined as a control experiment. It was found that PI-β-Ala scarcely induced the spectral change of PMTPA (Fig. S5, ESI†). These results indicated that sulfonate in the PI derivative does have a strong affinity for the quaternary ammonium group in PMTPA relative to carboxylate.

To check the performances of PMTPA toward sensing taurine, variation in the absorption spectra of PMTPA with increasing amounts of taurine in borate buffer (20 mM, pH = 9.0) was examined (Fig. 5). It was found that upon addition of increasing amounts of taurine, the absorption maximum is gradually red-shifted from 407 to 541 nm along with a dramatic color change from yellow to red–pink. The inset of Fig. 5 displays a linear relationship between A_{541}/A_{407} and the concentration of taurine (R = 0.995 from 0.01 to 0.40 mM), indicating that this approach is applicable to the ratiometric detection of taurine.[18]

In summary, we have confirmed that the charged moieties in monovalent inducent molecules play a crucial role in regulating the aggregation of cationic PMTPA. According to HSAB principle, soft–soft combination facilitates the primary electrostatic interaction between PMTPA and the inducent molecule (aromatic sulfonate) during the ionic self-assembly process, and further enhance secondary interactions including π–π stacking and hydrophobic interactions, thus promoting the formation of π-stacked PMTPA aggregates. We expect that the present findings will not only provide an important clue to understand the induced-aggregation mechanism of CPE, but also open a door to colorimetric sensing of structurally simple monovalent bioanions.

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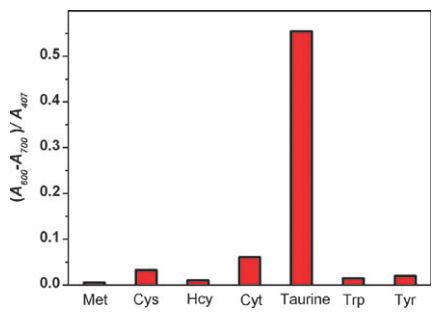

Fig. 4 Relative absorbance of PMTPA (1.0×10^{-4} M) in the presence of OPA (7.5×10^{-4} M) and Met, Cys, Hcy, Cyt and taurine in borate buffer (pH = 9.0): [amino acid] = 5.0×10^{-4} M.

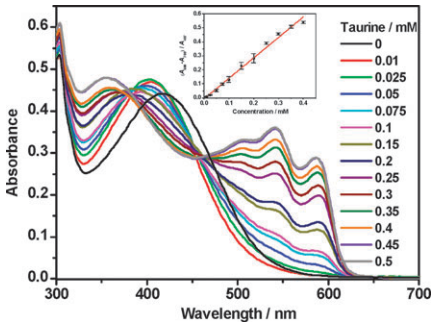

Fig. 5 Variation in the absorption spectra of PMTPA (1.0×10^{-4} M) in 20 mM borate buffer (pH = 9.0) with increasing concentrations of taurine as indicated: [OPA] = 1.0×10^{-3} M. Inset: the relationship between $(A_{600} - A_{700})/A_{407}$ and the concentration of taurine from 0.01 to 0.40 mM.

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18 The fluorescence method can extend the detection limit to the order of 10^{-8} M (see Fig. S7, ESI†).

