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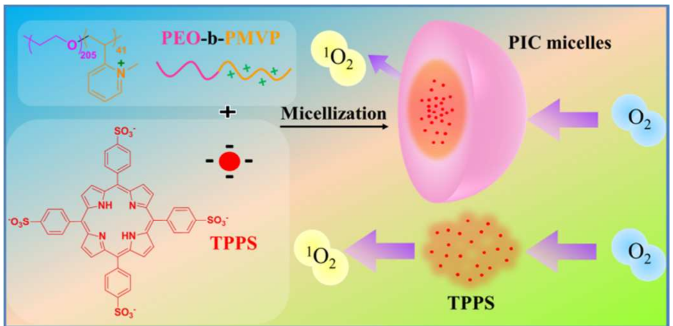
Graphical Abstract



The production of $^1O_2$ from TPPS can be reduced upon the formation of polyion micelles with $PMVP_{41}$-b-PEO_{205}.

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Suppress Singlet Oxygen Formation from 5,10,15,20-Tetrakis(4-sulfonatophenyl)porphyrin Using Polyion Complex Micelles

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Suppress the overproduction of harmful active oxygen species is very important. We report that the production of $^1O_2$ from TPPS can be reduced upon the formation of polyion micelles with $PMVP_{41}$-b-PEO_{205}. The amount of $^1O_2$ can be controlled successfully, which affords a new thinking of disease treatment and oxidation resistance of cells.

Polyion complex (PIC) micelles have attracted increasing attention in the field of macromolecular self-assembly, since they are found very useful in a wide variety of fields, such as controlled release,[1] transduction of gene,[2] sensors,[3] nanoreactors[4] and delivery of biomolecules.[5] Different from surfactant micelles with a simple hydrophobic core covered by hydrophilic head groups, the PIC micelles often have core-shell structures so that the micellar core is deeply buried in the forest of the corona.[6] This makes it difficult for small molecules to defuse into the micellar core, and the component that is enriched in the micellar core, such as DNA and proteins, can be well protected.[7]

Meanwhile, packing of small molecules such as porphyrin derivatives in the core of PIC micelles is also of interest since porphyrins play key roles in a wide variety of biochemical processes,[8] electronic devices[9] and biomimic chemistry.[10] For instance, Shi et al studied the aggregation and optical performance of the dye 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin (TPPS) in acidic aqueous media. They found that TPPS still retains the ability to form pH dependent H and J aggregates in the core of the PIC micelles formed with PEO-P4VP.[11] Considering that various porphyrin derivatives are relevant to many biochemical processes, especially in producing singlet oxygen ($^1O_2$),[12] it will be very interesting to study the effect of PIC micellization on the ability of producing $^1O_2$, preferably, in neutral aqueous solution, which is closer to physiological pH of living organisms.[13] Usually, $^1O_2$ is continuously produced in oxygen metabolism of living organisms, and controlling the quantity of $^1O_2$ in a normal level is very crucial for signal transduction, immune system control, cellular signalling, blood pressure modulation and the metabolism.[14] When overproduced or the levels of antioxidants become seriously depleted, the high quantity of $^1O_2$ may cause oxidative stress through the oxidation of biomolecules which can irreversibly damage the lipids of cellular membranes, proteins, enzymes, carbon hydrates or DNA. This may result in diseases and aging.[15] For instance, porphyria, one of disorder of porphyrin metabolism in organisms, is triggered by porphyrin accumulation which overproduces $^1O_2$ via irradiation leading to a series of harmful effects.

On the other hand, as the major bactericide, $^1O_2$ has significance in photodynamic therapy (PDT) to destroy tumors.[16] In this regard, many efforts have been made to enhance the amount of $^1O_2$ so that to make full use of the promising noninvasive cancer treatment approach of PDT.[17, 18] So far, there are no reports on how to reduce the amount of $^1O_2$ to avoid the harm of over expression on cells. Herein we show that the production of $^1O_2$ can be effectively deceased upon PIC micelle formation. This indicates that upon formation of PIC micelles, we can effectively tune the production of $^1O_2$ in a negative way, which may offer an important approach to suppress the harmful accumulation of $^1O_2$.

In this work, we first show the formation of PIC micelles with TPPS and $PMVP_{41}$-b-PEO_{205} block copolymer in neutral aqueous solution, then report the effect of PIC micelles on the production of $^1O_2$. The ability of antioxidant action and photostability of PIC micelles endows them with potential applications in treatment of the diseases triggered by the harmful accumulation of $^1O_2$. Finally, we show that the production of $^1O_2$ can be controlled by tuning the intactness of the PIC micelles with addition of NaCl to shield the charge interactions. We believe that a controllable $^1O_2$ generation would afford the base and a new thinking of PDT.

In neutral aqueous solution, TPPS molecules are tetra-anionic and exist in the form of monomeric free base (H_2TPPS^{4-}) due to electrostatic repulsion between H_2TPPS^{4-}.[19] Upon mixing with the positively charged double hydrophilic block copolymer $PMVP_{41}$-b-

PEO_{205}, strong Tyndall effect was observed (Inset in Fig. 1a), suggesting the formation of polyion complex micelles, which was confirmed later with TEM observations (Fig. 2).

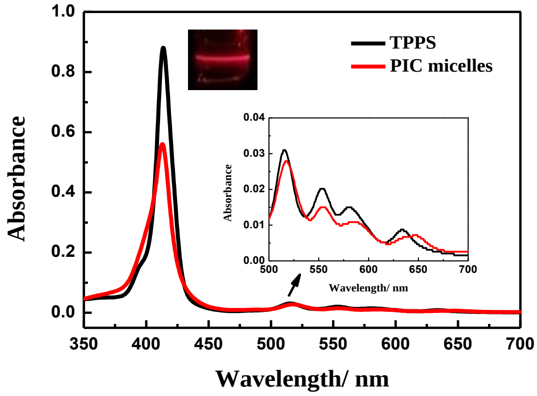

Fig. 1 UV-vis absorption spectra of the TPPS and PIC micelles in neutral aqueous solution.

The formation of micelles leads to significant change of the UV-vis absorption of TPPS. The black line in Fig.1a displays the UV-vis spectrum of TPPS in neutral aqueous solution. The characteristic features of an intense Soret band at 413 nm and four weak Q bands at 515, 553, 580 and 634 nm are observed. Upon formation of micelles, the Soret band of TPPS was significantly reduced by a ratio of ca. 30%. Meanwhile, the Q bands were shifted to 517, 554, 584 and 647 nm, respectively. These results suggest that the formation of PIC micelles have changed the local environment of TPPS, but the TPPS molecules in the micellar core still exist in the form of monomers. Namely, no H or J aggregates of TPPS are formed in the micelles, which is different from the behaviours in acidic solutions.[20]

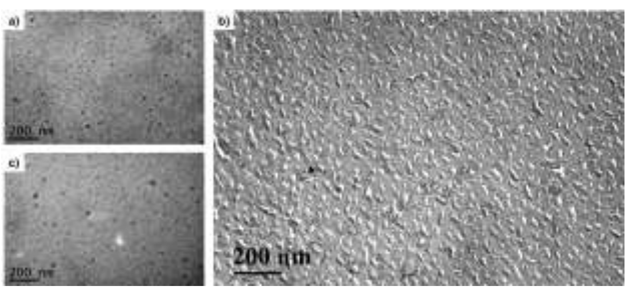

Fig. 2 TEM images of PIC micelles. (a) and (c) are the micelles without and with 0.3 M NaCl; (b) FF-TEM micrograph of micelles without NaCl. The concentration of TPPS and the charged unit MVP in $PMVP_{41}$-b-PEO_{205} is 20 and 80 μM, respectively, to reach charge balance between the negative and positive charges.

The formation of micelles also results in disappearance of the proton signals of TPPS in the ^1H NMR spectrum. Fig. S1 shows that before interaction with $PMVP_{41}$-b-PEO_{205}, all the protons of TPPS are discernible, but signals for these protons were silenced in the presence of $PMVP_{41}$-b-PEO_{205}, suggesting the TPPS molecules have been packed into the micellar core.[11] TEM observation indicates the average diameter of the micellar core is about 12 nm (Fig. 2a), which is in accordance with other PIC micelles formed with $PMVP_{41}$-b-PEO_{205}. Unfortunately, the overall hydrodynamic radius of the micelles can hardly be obtained with dynamic light scattering (DLS) due to the strong absorbance of 500-650 nm laser by TPPS in neutral solution. However, the thickness of the protecting PEO corona can be estimated from the TEM images by measuring the closest distance between two neighbouring micellar cores. This value is about 15~20 nm, which is also in good agreement with our previous report.[21, 22] Furthermore, the overall structures of the PIC micelles formed with TPPS and $PMVP_{41}$-b-PEO_{205} are fluid-like, so that irregular morphology was obtained when the sample was subjected to free-fractured TEM observations (Fig. 2b). The average size for the micelles obtained in FF-TEM is about 40-50 nm, which is in line with the estimation with previous.

Porphyrins are well-known for their ability of generation of $^1O_2$, which is caused by the transference of energy to triplet oxygen from triplet state porphyrin photosensitizer induced by light. To evaluate the activity of the photosensitizer of TPPS, we employed the "iodide method" to detect the formation of $^1O_2$. The principle behind this method is that the amount of I^{3-} produced by oxidation of I^- with $^1O_2$ is proportional to the concentration of $^1O_2$ under continuous irradiation.[23] Therefore, the UV absorption of I^{3-} can be used to track the production of $^1O_2$.

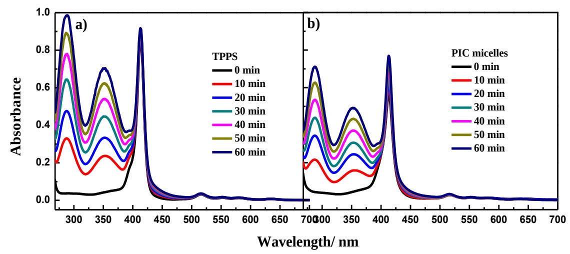

Fig. 3 UV-vis absorption spectra in dependence on irradiation time for (a) TPPS and (b) the PIC micelles; both in iodide solution with c(iodide) = in 0.05 M.

Therefore, we recorded the UV spectra changes of TPPS in the presence of KI before and after the PIC micelle formation, respectively (Fig. 3), under continuous UV irradiation. The bands at λ = 353 nm and λ = 287 nm are the characteristics of absorption from tri-iodide. Fig. 3a shows that these bands keep increasing with prolongation of UV irradiation time, suggesting the accumulation of $^1O_2$ in the free TPPS solution. It is evident that these bands get much lower in the PIC micelles than those of free TPPS. Hence, the formation of PIC micelles indeed suppressed the production of $^1O_2$ (Fig. S2). In Fig. 4, we compared the absorption at λ = 353 nm at different solution environment. It is found that the absorption was reduced by a ratio of about 30% in the micelles. Since the absorption of tri-iodide is proportional to the production of $^1O_2$, this indicates that PIC micelles can suppress the $^1O_2$ formation by a factor of about 30%. This value is rather significant in terms maintaining the level of $^1O_2$ in a normal level. This means that lower efficiency of producing $^1O_2$ is indeed realized by entrapping the porphyrins into the core of PIC micelles. We expect that this decreases the possibility of energy transformation from porphyrin to oxygen molecules to produce $^1O_2$.[24]

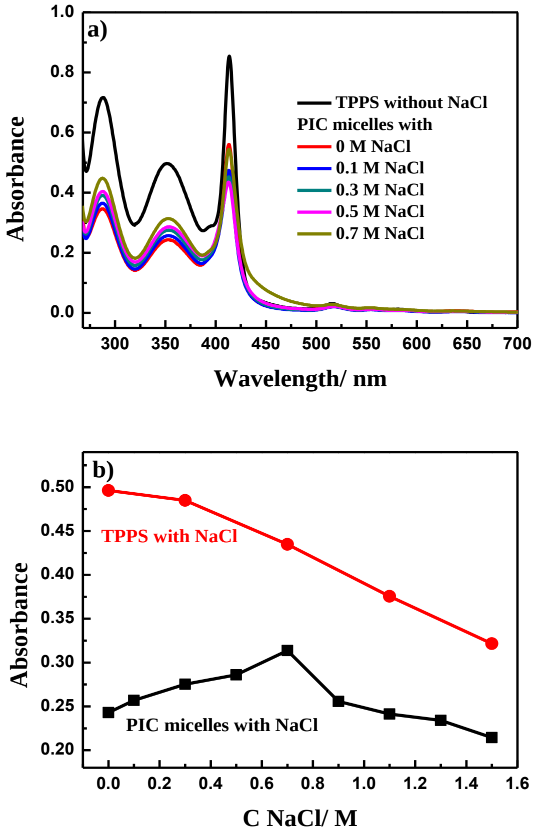

Fig. 4 (a) UV-vis absorption spectra of TPPS and the PIC micelles in dependence on NaCl concentration (b) the iodide absorption at λ = 353 nm of TPPS and the PIC micelles in dependence on NaCl concentration; both in iodide solution with c(iodide) = in 0.1 M and for 10 min irradiation time.

In order to gain more physical insight about the reduced production of $^1O_2$, NaCl was added to the PIC micellar system to disintegrate the micelles. It is well known that the electrostatic complex micelles driven by electrostatic interaction are sensitive to ionic strength. Then the increase in ionic strength of the solution could diminish the driving force for micellization by shielding the electrostatic interactions between TPPS and $PMVP_{41}$-b-PEO_{205}.[25] It was found that with increasing the salt concentration the absorption indeed partly recovered, suggesting the disassembly of micelles helps the production of $^1O_2$. We noticed that the level of $^1O_2$ never completely resumed to that in the free TPPS system. Even the concentration of NaCl is as high as 0.7 M (Fig. 4a), where most the PIC micelles were broken (SI, Fig. S2b), the production of $^1O_2$ is still much lower than that in the free TPPS system. Further increase of the concentration of NaCl resulted in a decrease in the absorption again (Fig. 4b). It should be noted that the addition of appropriate NaCl didn't change the characteristic UV absorbance of TPPS (Figure 4a and SI Figure S4), and the micelles are completely broken in the presence of > 0.7 M NaCl (Fig. S2c). Control experiments revealed that the presence of NaCl may decrease the production of $^1O_2$ either. But the ability of small ions in this regard is very limited, which only becomes significant at salt concentration beyond 0.7 M. Especially, in practical applications, the ionic strength in biological environment corresponds only to about 0.15 M NaCl. At this low salt concentration, the suppression of $^1O_2$ by NaCl is negligible, and the contribution of PIC micelles is dominant.

Conclusions

In conclusion, the approach of polyion complex (PIC) micelles is verified effective in suppression the overproduction of $^1O_2$ from anionic 5, 10, 15, 20-tetrakis(4-sulfonatophenyl)porphyrin (TPPS). The production of $^1O_2$ can be reduced by a factor of 30% via the formation of the PIC micelles. This factor is very significant in keeping the level of $^1O_2$ in a normal level. We believe that this work would be helpful in understanding the characteristics of bio-aggregates and the practical treatment of diseases triggered by harmful accumulation of $^1O_2$.

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Notes and references

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† Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/c000000x/

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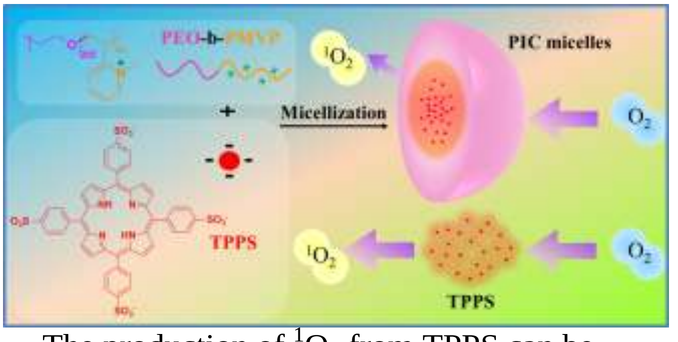

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