RSC Advances

ROYAL SOCIETY OF CHEMISTRY

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Cite this: DOI: 10.1039/c0xx00000x

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ARTICLE TYPE

Redox-controllable self-assembly and anti-bacterial activity of a vancomycin derivative

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

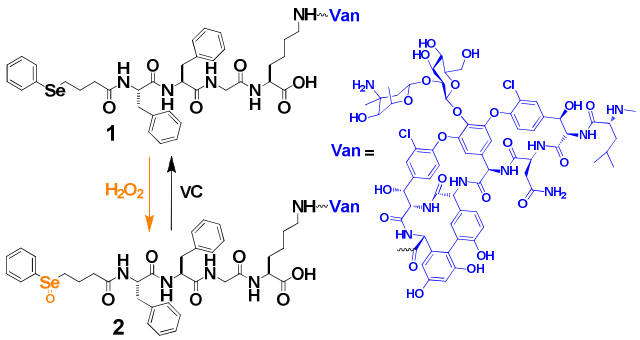
DOI: 10.1039/b000000x

We reported on a selenium containing vancomycin derivative with redox-controllable self-assembly property and anti-bacterial activity.

Inspired by the self-assembly systems in biological systems (e.g. actin filament or tubulin assembles), researchers have developed many nanostructures with controllable and reversible self-assembly properties, which are generally achieved by redox,[1, 2] light irradiation,[3] ligand-receptor interaction,[4, 5] etc. These nanomaterials can not only mimic and help to understand the natural self-assembly systems, but also show big potential in drug delivery[6] and tissue engineering.[7] Among the building blocks for constructing these nanomaterials, those of small molecules have attracted increasing attentions recently due to the ease of design and synthesis, good biocompatibility, and easy degradability of small molecules.[8] One strategy to generate reversible self-assembling small molecules is the integration of redox-active moieties with self-assembling small molecules. For instance, the introduction of ferrocenoyl group,[9] ruthenium complex,[10] or selenium containing group[1] to self-assembling short peptides have yielded supramolecular nanofibers and hydrogels with responsive properties. These studies highlight the advantages and versatility of redox-active compounds in the generation of reversible self-assembling systems.

The emergence of vancomycin resistance enterococci (VRE) has caused serious problems[11] and the development of multivalent vancomycin (Van) has been demonstrated to be a powerful strategy to treat VRE, because multivalent Van derivatives show greatly enhanced binding affinities to bacterial cell wall peptide on VRE (e.g. D-Ala-D-Lactate).[12] For example, a self-assembling Pyren-Van conjugate could efficiently inhibit both gram positive bacterial and VRE[5] due to the enhancement of local concentration of the antibiotics.[13] Based on this information and our previous development of selenium containing self-assembling peptides[1], we opted to develop selenium containing Van derivative with redox-controllable self-assembly property and anti-bacteria activity.

As shown in Scheme 1, we firstly designed compound ***1*** as the selenium containing Van derivative, the selenium group can be oxidized to selenoxide by H_2O_2, leading to the formation of compound ***2***. The compound ***2*** can be transformed back to compound ***1*** by the vitamin C (VC). As many peptide derivatives with the amino acid sequences, FF or FFY have been demonstrated to possess excellent self-assembly property,[14] compound ***1*** and ***2*** might self-assemble into different kinds of nanosrtructures. The synthetic route for the compound ***1*** was described in Scheme S-1. In brief, after successfully preparing 4-(phenylselanyl)butanoic acid, it was then directly used for solid-phase peptide synthesis (SPPS) to produce $PhSe(CH_2)_3COFFGK$. The carboxyl group of Van was then conjugated with the amine group on the side chain of lysine (K) by a condensation reaction. Pure compound ***1*** was obtained by high-performance liquid chromatography (HPLC). Treating compound ***1*** with H_2O_2 (30%, 2 equiv. to compound ***1***) could successfully acquire compound ***2***. Compounds ***1*** and ***2*** could be dispersed in phosphate buffered saline (PBS, pH 7.0) at concentrations lower than 0.05 (0.5 mg mL^{-1}) and 0.1 wt % (1 mg mL^{-1}) respectively. The results revealed that the oxidized compound ***2*** was more soluble than the reduced compound ***1*** in aqueous solutions, which was consistent with our previous results.[1]



Scheme 1. Chemical structures of the selenium containing vancomycin (Van) derivatives and redox-triggered transformation.

After obtaining the two designed compounds, their controllable and reversible self-assembly property was analyzed by liquid chromatography mass spectrometer (LC-MS). The LC-MS results (Fig. 1) showed that compound ***1*** was gradually converted to compound ***2*** upon the addition of H_2O_2, and the conversion reached the equilibrium after about 6 hours with a conversion percentage of 96.1% (Fig. S-6). We then added 4 equivalent of VC (dissolved in PBS at 37 ^oC) to the resulting solution. Compound ***2*** could be completely

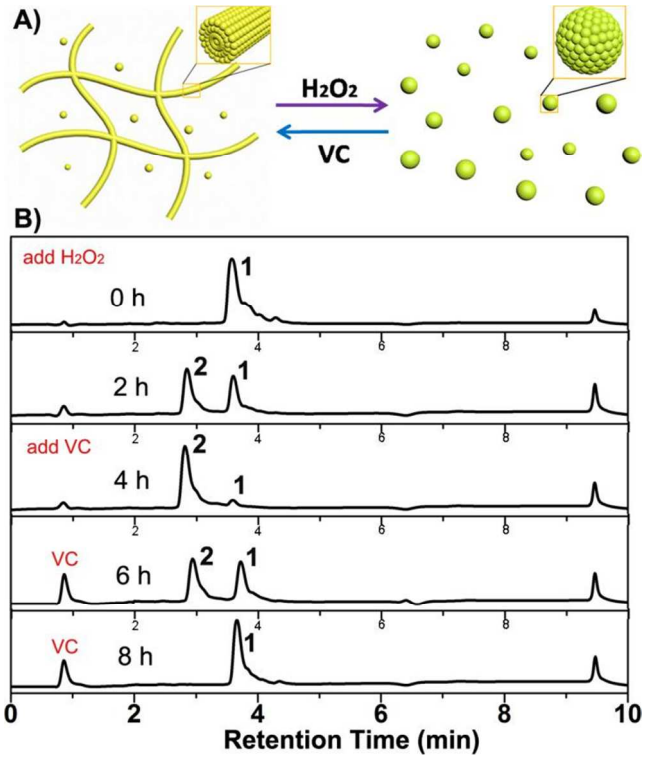


Fig. 1. A) Illustration of the redox-triggered transformation between micelles and nanofibers; and the HPLC spectra of one redox cycle triggered by H_2O_2 and VC.

transformed back to compound ***1*** after about 4 hours (Fig. 1 and Fig. S-8). We also found that this redox cycle could be repeated for at least three times without obvious decomposition of the compounds. Biocompatible triggers for the formation of nanostructures have recently attracted great research interests due to their big potential for the *in vivo* application.[15] The use of biocompatible agent VC to trigger the formation of nanostructure might advance the application of supramolecular nanostructures in tissue engineering, regenerative medicine, and controlled drug release.

We then used dynamic light scattering (DLS) and transmission electron microscopy (TEM) to characterize the self-assembly property of compounds and self-assembled nanostructures, respectively. A light beam could be clearly observed when PBS solution of compound ***1*** or ***2*** was shined by a laser pointer (insets in Fig. 2A and 2B), suggesting that both compounds ***1*** and ***2*** could self-assemble into nanostructures. The DLS results showed that the critical micelle concentration (CMC) of compound ***1*** and ***2*** were 25 and 542 μg/mL, respectively (Fig. 2A and 2B). The smaller CMC value of compound ***1*** indicated that compound ***1*** with reduced state have better self-assembly ability than compound ***2***. TEM images revealed that compound ***1*** exhibited nanofibers with a diameter of about 25 nm (Fig. 2C). Upon the addition of 2 equivalent of H_2O_2, the nanofibers changed to micelles with the size of about 10 nm (Fig. 2D). In order to test the reversible self-assembly property of the nanostructure, we then added 4 equivalent of VC to the resulting solution. The TEM image indicated that the micelles could be converted back to nanofibers (Fig. S-9). The phenomenon indicated that the transformation between micelles of compound ***2*** and nanofibers of compound ***1*** was switchable by the redox control.

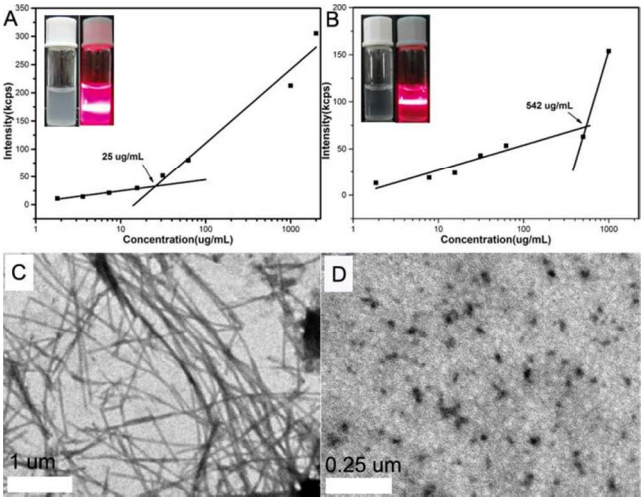


Fig. 2. The curves used to determine the CMC values of A) compound ***1*** and B) compound ***2***; insert in A) and B) displayed the optical image of ***1*** and ***2*** dissolved in PBS without and with shining by a laser pointer; and TEM images of C) the PBS solution of ***1*** in PBS (0.1 wt%) and D) the PBS solution of ***2*** resulting from the treatment of ***1*** with H_2O_2 (2 equiv; image taken after 24 h treatment).

We then studied the bacterial inhibition capacity of compounds ***1*** and ***2*** with different self-assembly properties. Two bacteria strains, Van sensitive strain of *Bacillus subtilis* and Van resistant enterococci (VRE) of *enterococus faecallis*, were chosen as model organisms. Standard broth microdilution assay was used to investigate the antibacterial activity of compounds ***1*** and ***2***. As shown in Fig. 3, for *Bacillus subtilis*, the minimum inhibition concentration (MIC) of compounds ***1*** and ***2*** were about 7.8 and 7.3 μM, respectively, which was comparable to that of parent Van molecule (3.5 μM). For VRE *enterococus faecalis*, the MIC values of compound ***1***, ***2*** and the parent Van were 850, 62.5,

and 250 μM, respectively, indicating that oxidative Van derivative compound ***2*** possessed the best inhibition capacity to the VRE *enterococus faecalis*. For a solution containing 400 μM of compound ***1***, it could not inhibit the growth of VRE *enterococus faecallis*. However, in the presence of 2 equiv. of H_2O_2, the solution could inhibit the growth of the VRE bacteria. Such switchable anti-bacteria activity was similar to those of biological systems that use kinase/phosphatase to regulate their activity.[16] The better inhibition capacity of compound ***2*** to VRE *enterococus faecallis* than compound ***1*** in nanofiber form was probably due to the higher surface areas of micelles than nanofibers at the same concentration. This observation was also consistent with our previous results that the catalytic activity of artificial esterase in nanospheres was higher than that in nanofibers. These results provided useful information to design nanomaterials with controllable bioactivities.

In summary, we reported in this study on selenium containing Van derivatives with redox-controllable self-assembly property and anti-bacteria activity. The transformation between self-assembled nanostructures is reversible and the nanostructures can be regulated by redox triggers. The different stages of the self-assemblies exhibited different bacterial inhibition capacities. Therefore, the anti-bacterial activity of the compounds can be controlled and is switchable. Our system of selenium-containing peptides with reversible transformations between different kinds of nanostructures or between dissociated and self-assembled stages could be used to switch and control the activity of bioactive molecules. We believe that this study will lead to the development of nature-mimicking smart materials with promising properties.

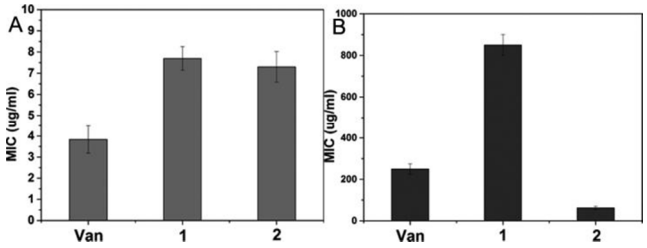


Fig. 3. Minimum inhibition concentration (MIC) of Van and compounds ***1*** and ***2*** to inhibit A) *Bacillus subtilis* and B) VRE *enterococus faecallis* investigated by standard broth microdilution assay

This work is supported by National Natural Science Foundation of China (31370964, 51203078, and 51173060), Tianjin MSTC (12JCYBJC11300), and Program for Changjiang Scholars and Innovative Research Team in University (IRT13023).

Notes and references

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*†Electronic Supplementary Information (ESI) available: [synthesis and characterization of the compounds, LC-MS results of the conversion between compounds **1** and **2**, TEM image of solution of **2** treated with VC, details experimental procedure of MIC assay]. See DOI: 10.1039/b000000x/*

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