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Synthesis and ultrastructural characterization of ferrocenylated soft structures

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ABSTRACT

A C_3-symmetric ferrocenylated ditryptophan construct was synthesized and the morphology of its self-assembled structure was studied. Ferrocenylated soft structures so obtained interacted with cyclodextrin resulting in increased tryptophan fluorescence and disruption of the self assembled structure.

Concepts concerning biological or bioactive compounds, where at least one carbon is directly bound to a metal center, lie in the domain of bioorganometallic chemistry. The application of such compounds encompasses fields as diverse as crystal engineering, catalysis to chemotherapeutics and this widespread potential makes such compounds highly worthy of intense scrutiny and exploration.[1] Ferrocene remains one of the most extensively studied organometallic compounds that have been conjugated to amino acids or peptides for a variety of applications.[2] Due to its well studied redox properties combined with suitable chemical reactivity, the ferrocenyl group is a remarkable building block for the construction of redox-responsive bioconjugates.

Ferrocene conjugation to polymers and polyelectrolytes, to afford formation of novel materials, is an area of contemporary interest.[3] Recently, redox-active properties of ferrocene were harnessed in a study related to aqueous phase self-assembly of polyferrocenylsilane–polydimethylsiloxane block vesicles, which led to the formation of organometallic vesicles.[4] In another study, mesoporous silica surface was modified with the ferrocene moiety which was cleaved with the help of ultrasound irradiation thus controlling the release of encapsulated material.[5]

Herein, we report a 'bottom-up' synthetic approach for the construction of ferrocenylated soft spherical structure via solution-phase self-assembly process. This synthetic approach is based around a ditryptophan triskelion core that forms caged structures in methanol–water.[6] The conjugation of ferrocene was chosen to eventually explore the electrochemical properties of self-assembled peptide-based structures and the possibility of tuning morphology by ferrocenyl pendants. Using an alternate synthetic strategy, we describe synthesis of **3** as a ferrocene functionalized scaffold for 'bottom-up' construction of soft structures. Scheme 1 shows stepwise synthesis of compound **3** where ferrocene carboxylic acid was coupled to ditryptophan dipeptide in a step-wise fashion, followed by conjugation of its active *N*-hydroxysuccinimide ester with tris(2-aminoethyl)amine (Tren), to afford the target compound **3**, after deprotection.[7]

Compound **3** (0.5 mM) was incubated in methanol at 37 °C for the microscopic studies. The spherical morphology was found to form within a minute of incubation in solution (see Supplementary data). Scanning electron microscopic image shows the spherical structure and magnified view of the surface shows that surface composed by assemble or fusion of the particles, with an average diameter of ~25 nm (Fig. 1a and b).[8] Similarly, AFM image also indicates the appearance of spherical structures and its surface analysis confirms fusion of the particles around 25 nm (Fig. 1c and d).[7] It is likely that two indole moieties of ditryptophan motif are stacked thus invoking favorable aromatic interactions, leading to interdigitation of three ditryptophan arms resulting in the formation of self-assembled soft spherical structures.[6]

Intrinsic fluorescence property of the tryptophan residues prompted us to determine fluorescence behavior of **3** in methanol/water (9:1) system. Interestingly, **3** did not exhibit any fluorescence compared to its nonferrocenylated analog $(TrpTrp)_3Tren$ (see Supplementary data), perhaps due to the reason that the ferrocenyl groups in each arm are oriented to interact with the π–π stacked indole units. It is possible that this occurs due to resonance energy transfer with Trp acting as a donor and a proximal ferrocene acting

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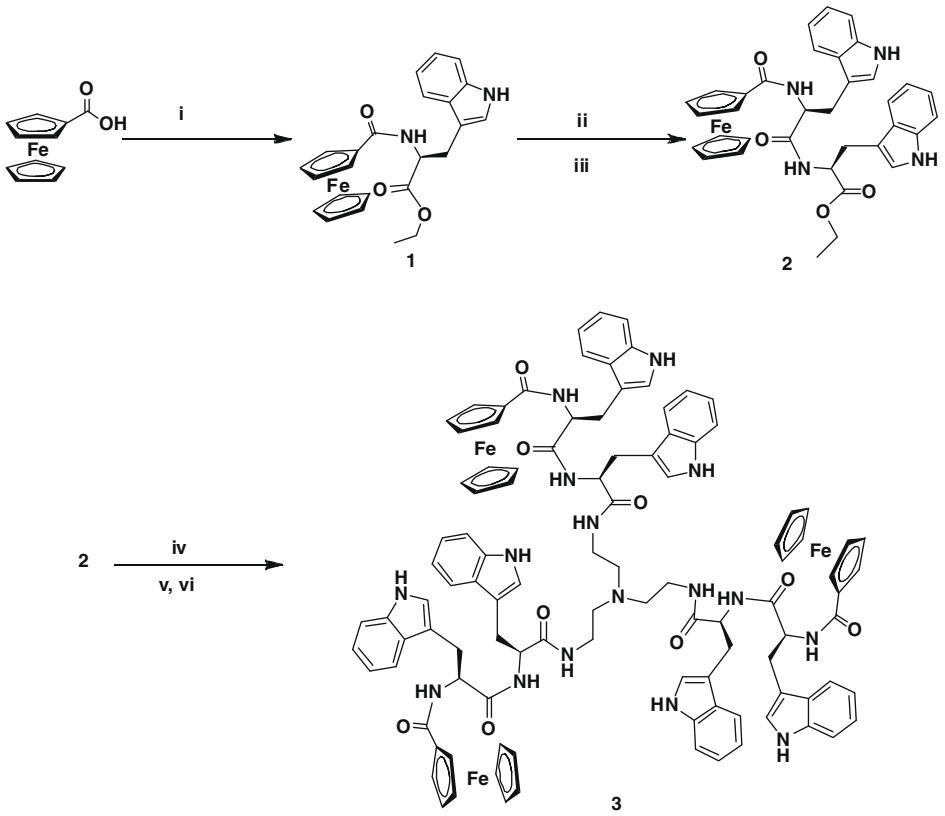

Scheme 1. Synthetic route for compound **3**. Reagents and conditions: (i) tryptophan ethyl ester hydrochloride, DCC, HOBT, 0 °C to rt, 24 h; (ii) 1 N NaOH, MeOH, 3 h; (iii) tryptophan ethyl ester hydrochloride, DCC, HOBT, 0 °C to rt, 24 h; (iv) 1 N NaOH, MeOH, 3 h; (v) DCC, *N*-hydroxysuccinimide, DMF, 0 °C to rt; (vi) Tren, DMF, rt, 24 h.

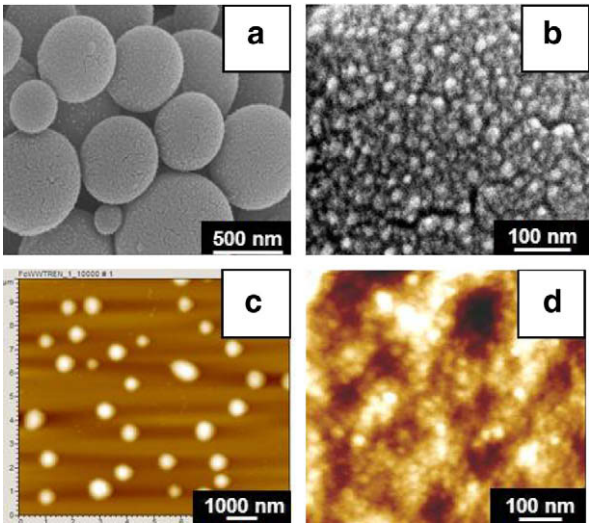

Figure 1. Ultrastructural details of **3**; (a) SEM image of the vesicles; (b) magnified image of the spherical surface; (c) AFM image of the vesicles; (d) magnified AFM micrograph of the surface.

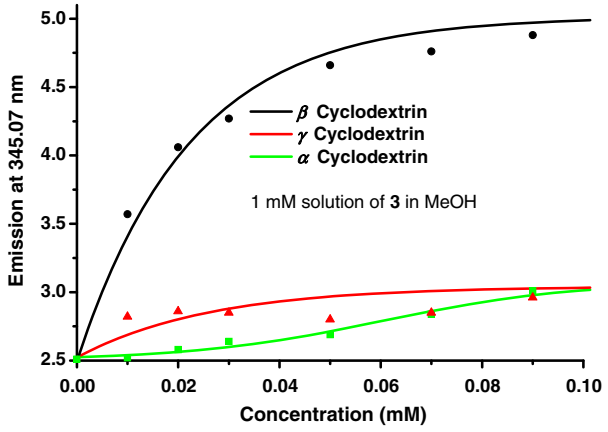

Figure 2. Effect of cyclodextrins on fluorescence behavior of compound **3**.

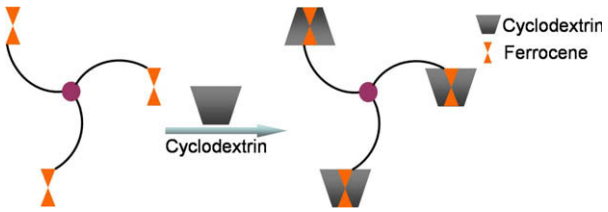

Figure 3. Graphical representation of ferrocene complexation by β-CD.

as an acceptor. Such proximity driven Trp fluorescence quenching has been reported for ferrocene-modified glucose oxidase conjugate.[9] In this study, extensive quenching of tryptophan fluorescence was observed for covalently modified conjugate, but not for the noncovalent mixtures containing ferrocene and glucose oxidase in variable stoichiometries, suggesting proximal interaction of donor/acceptor in the protein fold. On similar lines, we propose that tryptophan–ferrocene interaction in the composite soft supramolecular ensemble of **3** is implicated for fluorescence quenching. On the other hand, electron transfer between ferrocene and tryptophan residue also remains an intriguing alternative explanation for the fluorescence behavior. In this vein, a study by Faraggi et al. has

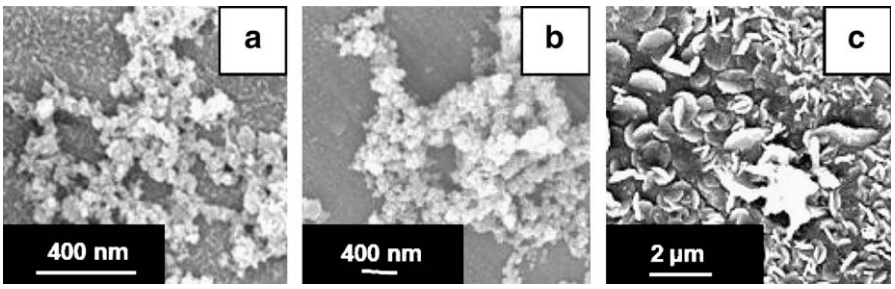

Figure 4. Morphological change in soft structures after addition of cyclodextrins: (a) α; (b) β; (c) γ.

previously described electrochemical study between tryptophan and a ferrocene dicarboxylic acid derivative.[10]

It is known that β- and γ-CDs form a 1:1 complex with ferrocene, while α-CD prefers a 2:1 stoichiometry of complexation.[11] Moreover, the orientation of ferrocene complexation in the β-CD cavity is considered to be nearly parallel to its molecular axis, while the complexation of ferrocene is nearly perpendicular to the molecular axis of γ-CD.[12] Thus, we decided to probe the orientation of appended ferrocenyl moieties by performing fluorescence experiments in the presence of α-, β-, and γ-cyclodextrins (CD). It was assumed that a suitable interaction of ferrocene and appropriate cyclodextrin will result in an increase in the Trp fluorescence due to the interference with Trp–ferrocene interaction. The concentration of CDs was gradually increased in methanolic solution of **3** (1 μM), and it resulted in fluorescence enhancement in all the three cases. However, the maximum fluorescence enhancement was observed for β-CD, while α- and γ-CDs afforded marginal enhancement (Fig. 2). Although, β- and γ-CD exhibit identical affinities for compound **3** but the greater fluorescence enhancement in the case of β-CD may arises from a more optimal orientation of the ferrocenyl moieties in the CD cavity.[13] It can be proposed that favorable host–guest interaction may alter the relative orientation of the interacting dipole moment of the ferrocenyl and tryptophan units, thereby inhibiting Förster resonance energy transfer, thus maximizing fluorescence enhancement.

The interaction of ferrocene with β-CD is also supported by the ^{1}H NMR shifts where shielding of ferrocenyl protons was observed upon increasing the concentration of β-CD (see Supplementary data). A schematic model is proposed on the basis of these results and the preferred directionality of ferrocene–β-CD interaction (Fig. 3).

Inclusion complexation of ferrocene inside cyclodextrin cavity modulates solution properties of ferrocene functionalized polymers due to disruption of hydrophobic interactions.[14] In another example, it was shown that cyclodextrin microgels loaded with guest molecules get disrupted in the presence of methyl orange solely driven by 'host–guest' inclusion effects.[15] Interestingly, we also observed that the morphology of conjugate **3** changes after addition of α-, β-, and γ-cyclodextrins. Spherical structure of the conjugate **3** disrupts to a random aggregate-like structure (Fig. 4), indicating a strong solution phase interaction between α-, β- and γ-cyclodextrins and conjugate **3.** We envisage that cyclodextrins act as a morphological switch for these soft structures by invoking 'host–guest' complexation effects.

Well known electroactive properties of ferrocenyl moiety suggests that self-assembled soft structures of **3** may afford interesting electrochemical behavior.[16] Preliminary electrochemistry studies indicated unusually high peak current associated with the oxidation and reduction process for **3** (data not shown). This may be ascribed to the adsorption of composite soft structures, whose surface is decorated by indole moieties and ferrocene, on the electrode surface. Detailed electrochemical and current sensing experiments will be reported elsewhere.

In conclusion, we have reported synthesis of a ferrocene–dipeptide conjugate and its application for the 'bottom-up' synthesis of soft spherical structures. The fluorescence property of the conjugate and the effect of cyclodextrin on the fluorescence property as well as morphology were studied. Loss of structural integrity upon cyclodextrin interaction and electroactive nature of these vesicles provides a convenient entry into organometallic supramolecular ensembles which can be harnessed for host–guest chemistry and material applications.

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Supplementary data

Supplementary data (experimental procedures, detail of microscopic studies, additional fluorescence data, ^{1}H NMR titration data, additional microscopic images) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.12.019.

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7. **Synthesis of (Fc-Trp-Trp)$_3$Tren** (**3**). Compound **2** (0.32 g, 0.51 mmol) was dissolved in methanol (9 mL) and 1 N NaOH (1.0 mL) was added into the solution at room temperature. The reaction mixture was stirred for 3 h at room temperature. Reaction mixture was concentrated to remove the methanol completely under reduced pressure. The residue was acidified with 1 N HCl (5 mL) and extracted in dichloromethane (3 × 20 mL). Combined organic layer was washed with water followed by brine (20 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure to get the solid Fc-Trp-Trp-OH (0.28 g, 0.46 mmol, 90%).
Crude Fc-Trp-Trp-OH (0.25 g, 0.41 mmol) and *N*-hydroxy succinimide (0.052 g, 0.45 mmol) were dissolved in 1,2-dimethoxyethane (20 mL) and the reaction mixture was cooled to 0 °C under nitrogen atmosphere. Solution of DCC (0.092 g, 0.45 mmol) in 1,2-dimethoxyethane (2 mL) was added into the reaction mixture dropwise and reaction mixture was stirred for 2 h at 0 °C. After that reaction mixture was kept in freeze for overnight. Reaction mixture was filtered and filtrate was concentrated under reduced pressure. Solid was washed with diethyl ether and dried under high vacuum pump. Crude compound (0.28 g, 0.40 mmol) was dissolved in dry DMF (3.5 mL) at room temperature under nitrogen atmosphere. Solution of tris(2-aminoethyl)amine (16 μL, 0.11 mmol) in dry DMF (0.5 mL) was added into the reaction mixture dropwise under nitrogen atmosphere at room temperature. The reaction mixture was stirred at room temperature under nitrogen atmosphere for 24 h. The reaction mixture was concentrated under reduced pressure and dissolved

in DCM. Organic layer was washed with 1 N HCl (3 × 10 mL), 10% $NaHCO_3$ (3 × 10 mL) and brine (10 mL). Organic layer was dried over anhydrous sodium sulfate and concentrated to give crude compound **3**. The crude compound was purified through silica-gel column chromatography by using dichloromethane methanol (94:6) solvent system to give pure compound **3** (0.15 g, 0.078 mmol, 19%). mp = compound decomposes at 150 °C, R_f [10% methanol in dichloromethane] = 0.6, ESI-HRMS: $[M+H]^+$, Calcd ($C_{105}H_{103}Fe_3N_{16}O_9$): 1899.6142, Found: 1899.6146, $[\alpha_D^{25}] = +00.01$ [*c* 1.2 methanol]). ^1H NMR (400 MHz, CD_3OD, TMS, δ ppm): 2.83 (m, 6H); 3.08–3.14 (m, 6H); 3.30 (s, 6H); 3.33 (s, 6H); 3.76 (s,18H); 3.80–3.82 (m, 3H); 4.26 (m, 6H); 4.5 (m, 3H); 4.56–4.58 (m, 6H); 4.70–4.72 (m, 3H), 6.77 (m, 3H); 6.8–7.1 (m, 15H), 7.17 (m, 3H); 7.23–7.25 (d, 3H, *J* = 8.0 Hz), 7.31–7.33 (d, 3H, *J* = 8.0 Hz); 7.43–7.45 (d, 3H, *J* = 8 Hz); 7.63–7.65 (d, 3H, *J* = 8.0 Hz); ^{13}C NMR (100 MHz; CD_3OD, δ ppm): 28.5, 28.8, 38.6, 54.1, 55.9, 68.0, 70.1, 70.6, 70.8, 71.9, 75.6, 110.7, 111.1, 112.4, 112.6, 119.4, 119.9, 120.2, 122.5, 122.7, 124.8, 128.8, 137.9, 138.2, 173.6, 173.8, 174.2; FT-IR (KBr, cm^{-1}): 1629 (amide II); 1737 (amide I); 3281 (–NH str).

8. Sample preparation for microscopy: **SEM**: 20 μL of the incubated solution of conjugate **3** was coated atop metal slides. A gold coating was applied to the top of the sample to make it conductive for analysis. SEM micrograph of **3** after 1 min incubation in methanol is provided in *Fig. S2* the samples used was 0.5 mM in methanol. For the morphological disruption study, cyclodextrins (0.1 mM each) in 80% methanol/water were added to the incubated sample (24 h) of compound **3** (0.5 mM) in methanol. **AFM**: The mixture of the peptide solution was incubated for 0–7 days in methanol and micrographs were recorded for selected incubation periods. Ten microliters of sample (0.5 mM) solution was transferred onto freshly cleaved mica surface and uniformly spread with the aid of a spin-coater operating at 200–500 rpm (PRS-4000). The sample-coated mica was dried for 30 min at room temperature, followed by AFM imaging.

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