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Self-Assembled Morphologies from C_2- and C_3-Symmetric Biotin Conjugates

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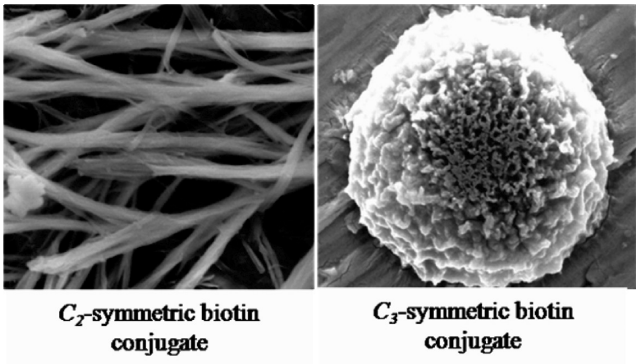

Synthesis and characterization of C_2- and C_3-symmetric D-biotin conjugates has been realized, followed by the investigation of solution state self-assembly behavior of these conjugates by various microscopy techniques. Interestingly, the C_2-symmetric conjugate affords the formation of fibrous structures, while the C_3-symmetric conjugate reveals the formation of marigold flower-like spherical morphologies.

Biotin is a water-soluble vitamin, which acts as a cofactor in a number of important biochemical metabolic reactions.[1,2] The structural characterization of biotin in the solid state has revealed an extended structure, while multiple conformational ensembles ranging from extended to folded states are preferred in solution.[3,4] Theoretical simulation studies suggest that biotin structure is highly flexible and interconverts between extended, semifolded, and folded states, in aqueous solution. These conformations are believed to evolve via water-mediated intramolecular hydrogen bonds between the ureido group and the side chain carboxyl, to exhibit cooperativity between various folded and semifolded states.

Molecular self-assembly provides a rapid entry into construction of nanoscale ensembles with potential applications in electronic, optical, magnetic, and biologically active materials.[5] The ability of nanoscopic components to self-assemble to form supramolecular superstructures critically depends on precise noncovalent interactions such as van der Waals, hydrogen bonding, electrostatic, magnetic, molecular, and entropic forces, which can be invoked to guide the molecular self-assembly process.[6] Of these, hydrogen bonding is one of the most widely used interactions to engineer self-assembly owing to their directionality, moderate strength, and the possibility of enhancing stability of ensembles by simply increasing the overall number of potential hydrogen bonding sites.[7] Hydrogen bonding as a stabilizing factor has been extensively applied for the design of self-assembling short peptides which could spontaneously organize into well-ordered nanoscale structures. These peptide nanofibers exhibit diverse applications in tissue cell culture, regenerative scaffolds for tissue engineering, sustained drug release devices, and in other areas of nanobiotechnology.[8]

The self-assembly of biotin and its conjugates is mainly attributed to the intermolecular hydrogen bonding between the ureido group and the valeryl carboxyl group.[3b,9] The donor−acceptor−donor (DAD) hydrogen bonding scheme of the ureido group is shaded to highlight its presence in biotin conjugates (Figure 1). Crystal structure studies have suggested involvement of a hydrogen bond in biotin-bicarbonate recognition through the ureido group,[10] which can also facilitate polarization of carbonyl oxygen in the ureido part of biotin.[11] Thus, it should be possible to invoke hydrogen bonding at the ureido group of biotin conjugates to create supramolecular ensembles in solution.

We have worked on a covalent linking strategy to maximize noncovalent interactions in self-assembling of peptides and peptide-conjugates, thus providing an expeditious entry

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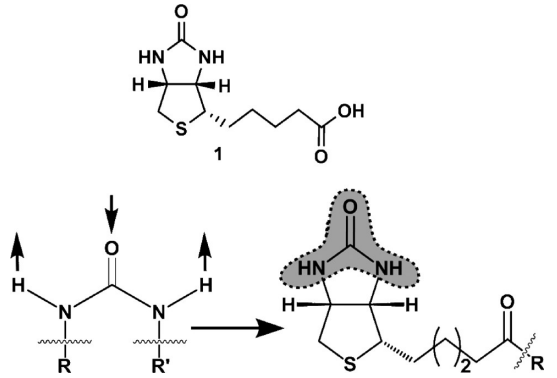


FIGURE 1. Top: Molecular structure of D-biotin (**1**). Bottom: Representation of DAD hydrogen bonding scheme around substituted urea and in particular the biotin ureido group.

to the generation of peptide-based soft structures.[12] One of our recent studies of peptide−biotin conjugate suggested the possibility of morphological trigger converting fibrous self-assembled structures into spherical aggregates.[12a] With this background, we decided to probe the solution-phase ultrastructure of free biotin **1** and its synthetic conjugates namely (biotin)$_2$-DAE (1,2-diaminoethane) **2**, and (biotin)$_3$-Tren **3** (Scheme 1).[13]

Biotin conjugates **2** and **3** were synthesized as described (Scheme 1).[13] 1,2-Diaminoethane (DAE) and tris(2-aminoethyl)amine (Tren) were employed as C_2- and C_3-symmetric linkers. These linkers not only serve as a scaffold for attaching biotin moieties, but are also expected to aid spatial directionality to the biotin ureido groups for further interaction in the solution state.

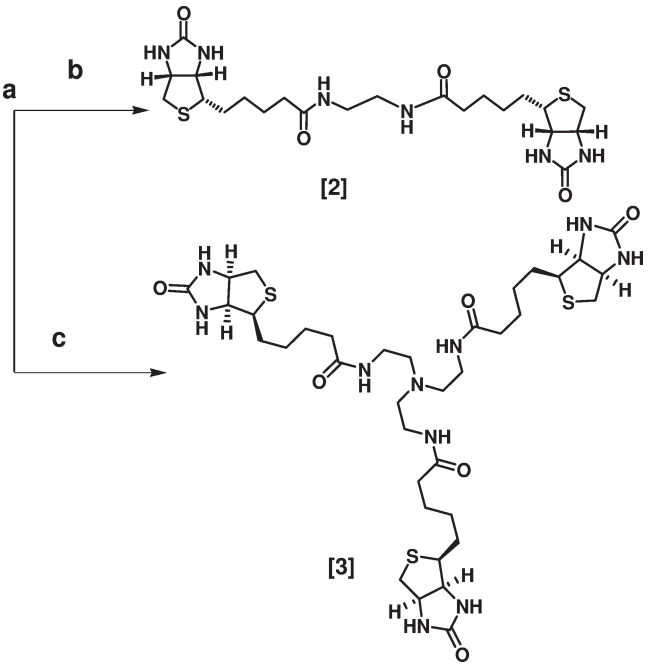
Scanning electron microscopic (SEM) analysis of a freshly prepared solution of D-biotin **1**, C_2-symmetric conjugate **2**, and C_3-symmetric conjugate **3** (1 mM, 50% aqueous methanol) was conducted. The self-assembly of a fresh sample of **1** revealed the formation of 1−5 μm long fibrous structures (Figure 2a).

Fresh sample of compound **2** revealed a micrometer long, straightened tubular fibrous network with a cross-section of ~0.5−1.0 μm (Figure 3a). Interestingly, the fresh sample of C_3-symmetric conjugate **3** afforded radial growth of fibrils, from a central core, forming a spherical morphology resembling a marigold flower (Figure 3b). Notably, three biotin pendants emerge from the Tren core in conjugate **3**.

A prolonged incubation of these conjugates leads to elongated structures with refined gross morphologies as detected by microscopic techniques. Flattened tubular morphology with tapered ends was observed for **2** (Figure 3c).

However, 5 days aged sample **3** almost retained the original morphology suggesting that self-assembly of **3** is almost instantaneous and stable and it does not appreciably evolve over a period of time (Figure 3d).

SCHEME 1. Synthetic Scheme for Conjugates 2 and 3[a]



[a]Reagents and conditions: (a) (+)-biotin-*p*-nitrophenyl ester, (b) DAE, DMF, N_2, 24 h, rt; and (c) Tren, DMF, N_2, 24 h, rt.

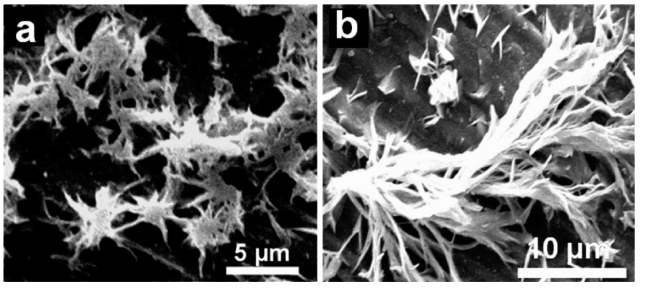


FIGURE 2. SEM micrograph of (a) fresh samples of **1** and (b) ~12 days aged samples of **1**.

Formation of self-assembled structures possessing diverse morphologies can be attributed to the bis linker such as 1,4-diaminobutane and tripodal scaffolding template such as Tren,[14] which aid in enhanced hydrogen bonding interactions via ureido, carboxyl, and other groups. In this context, the occurrence of dynamic changes in ^{1}H chemical shifts, confirming formation of intramolecular hydrogen bonds, has already been reported for various biotin derivatives.[15]

It was further possible to stain these structures with rhodamine B dye for fluorescence detection (Figure S1, Supporting Information, and Figure 4). A 1 mM solution of biotinylated conjugates and 10 μM rhodamine B was coincubated in 50% methanol/water solvent and a 20 μL aliquot of the aged solution was loaded on a glass slide, dried at room temperature, and imaged under a fluorescence microscope.

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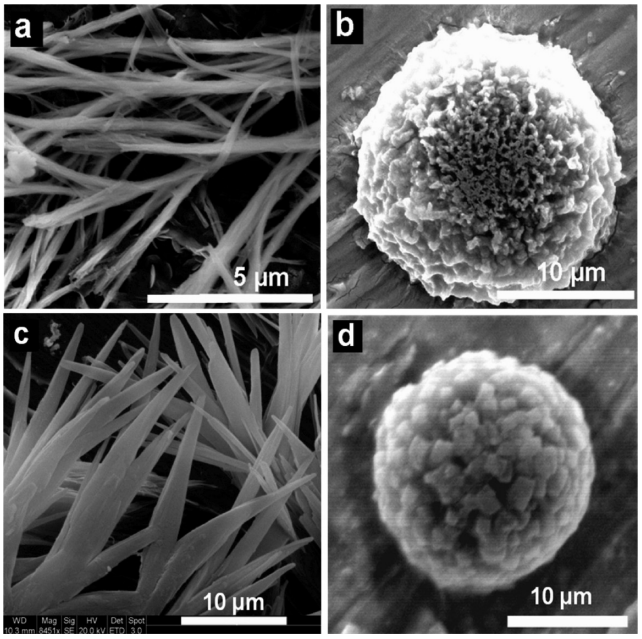

FIGURE 3. SEM micrograph of self-assembly D-biotin conjugates: fresh samples of (a) **2** and (b) **3** and 5 days aged samples of (c) **2** and (d) **3**.

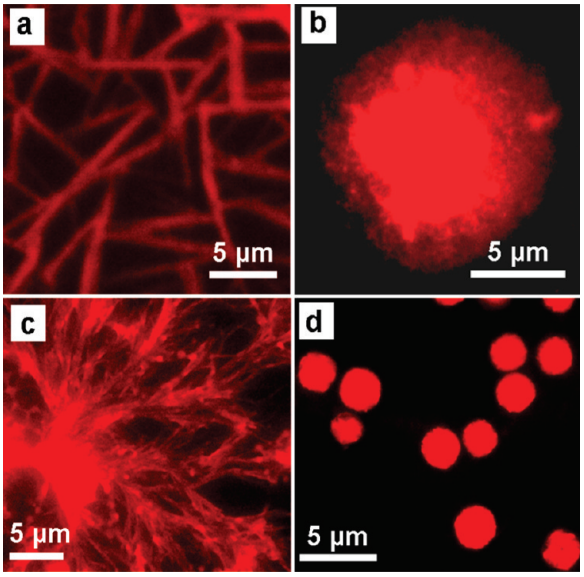

FIGURE 4. Fluorescence optical micrograph of self-assembly of D-biotin and its conjugates stained with rhodamine B. Aged sample (up to 5 days) of (a) **2** and (b) **3** and 30 days aged sample of (c) **2** and (d) **3**.

Rhodamine B stained microscopic data corresponded well with the gross morphologies found in SEM observations (Figure 4).

A freshly prepared solution of D-biotin **1** and derivatives **2** and **3** also revealed self-assembled morphology when studied with atomic force microscopy[13] confirming a time-dependent aggregation and fibrillation event (Figure 5). AFM micrographs of 12 days aged sample of **1** showed intensive fibrillation compared to the fresh one[13] and 5 days aged sample of **2** on mica surface revealed formation of long fibers of micrometer length scale and a cross-section from 0.5 to

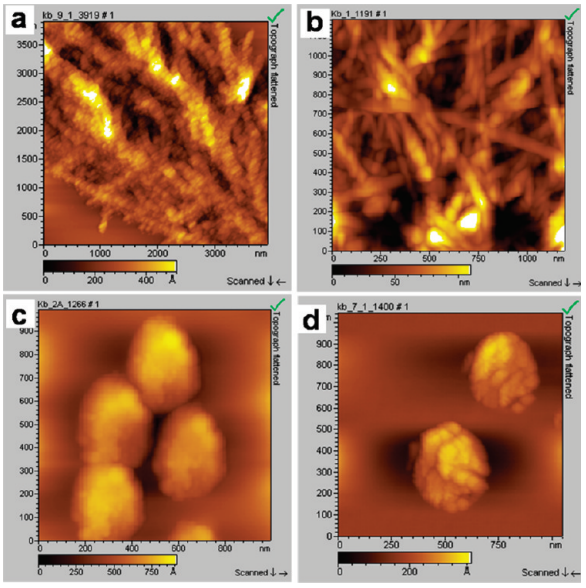

FIGURE 5. AFM micrograph of self-assembly of D-biotin and its conjugates. Twelve days aged sample of (a) **1**, 5 days aged sample of (b) **2**, and 5−10 days aged sample of (c, d) **3**.

1.0 μm (Figure 5b). Such a pattern was duplicated when the surface was changed to a more hydrophobic quartz surface.[13] On the other hand, 5 days aged conjugate **3** showed the formation of spherical self-assembled structures on mica (Figure 5c,d), graphite, and quartz surfaces.[13]

The propensity of hydrogen bonding between the biotin ureido group and the valeryl carboxylic acid side chain has been confirmed in solution as a well as solid state. The possible existence of fully extended to semifolded state structures of D-biotin and **2**, and hydrogen bonding lead to fibrous self-assembled structures in solution, while preliminary modeling studies with C_3-symmetric biotin conjugate suggests occurrence of stacking interactions along with hydrogen bonding leading to stable spherical aggregates. It is possible that the Tren scaffold also imparts certain directionality by disposing biotin units in different directions, thereby leading to a marigold flower-like self-assembly. Binding of water molecules to biotin conjugates might also add to the driving force behind this self-assembly process, in addition to the possibility of DAD hydrogen bonding. Water is known to bind biomolecules and is inextricably involved with their structure and biological function.[16] These water molecules can act as bridges between hydrogen bonding interfaces, such as ureido and valeryl side chain in biotin, or screen unfavorable electrostatic contacts, as determined by theoretical energetic contributions.[16a] Thus, it is possible that ordered water molecules surround biotin and a closer approach of biotin or its derivatives during the self-assembly process disrupts associated water−biotin interaction, releasing them in bulk water leading to an entropic gain. This stabilizing gain, coupled with formation of new hydrogen bonds in the bulk water phase and between self-assembling biotin moieties, could be expected to drive the process

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forward. A similar situation of water reorganization upon streptavidin−biotin interaction has been reported earlier.[17]

In conclusion, this study gives an interesting insight into the contributions of biotin and its symmetrical conjugates in providing driving force and stability to the self-assembly process. As biotin is used as an indispensable cofactor in many biochemical reactions,[1,2,18] the present studies suggest an intriguing possibility of the presence of various biotin superstructures in standing solutions which may aid or interfere with enzymology. Future work will involve mechanistic investigation of the self-assembly process and the use of these self-assembled structures in enzymatic transformations involving biotin as a cofactor.

Experimental Section

Details of sample preparation for microscopic analysis are presented in the Supporting Information.[13]

Synthesis of (Biotin)$_2$DAE (2). (+)-Biotin 4-nitrophenyl ester (200 mg, 0.54 mmol, 1 equiv) was dissolved in dry *N*,*N*-dimethylformamide (10 mL) at room temperature under nitrogen atmosphere. The 1,2-diaminoethane (14 mg, 16 μL, 0.24 mmol, 0.45 equiv) was dissolved in dry *N*,*N*-dimethylformamide (1.0 mL) followed by dropwise addition with the help of a micropipet into the reaction mixture under nitrogen atmosphere at room temperature and the reaction mixture was set aside for 24 h. The reaction was monitored by TLC. After the reaction was complete, the solvent was evaporated under reduced pressure and the residue was triturated with diethyl ether (3 × 10 mL). The crude solid product was washed with 1 N HCl (3 × 10 mL) and distilled water. The crude compound was purified through silica gel column chromatography with a dichloromethane methanol (80:20) solvent system to give pure compound [**2**] (163 mg, yield 58%). $[\alpha]^t_D$ +40 (*c* 0.1 DMF); R_f 0.4 in 20% MeOH−DCM; mp 87−90 °C; ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS), δ (ppm) 1.19−1.29 (m, 4H), 1.42−1.47 (m, 4H), 1.54−1.59 (m, 4H), 2.02 (t, J = 7.35 Hz, 4H), 2.55 (d, J_{gem} = 12.4 Hz, 4H), 2.76−2.77 (dd, J = 5.4, J_{gem} = 12.35 Hz, 4H), 3.03−3.12 (m, 2H), 4.09−4.10 (m, 2H), 4.26−4.28 (m, 2H), 6.33 (s, 2H), 6.39 (s, 2H), 7.77 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS) δ (ppm) 25.2, 27.9, 28.4, 35.3, 39.2, 39.9, 55.4, 59.2, 61.0, 162.8, 172.3; HRMS $[M + Na]^+$ for $C_{22}H_{36}N_6O_4S_2Na$ calcd 535.2137, obsd 535.2137.

Synthesis of *N*,*N*′,*N*″-Tris[*N*-biotinyl](2-aminoethyl)amine (3). (+)-Biotin 4-nitrophenyl ester (80 mg, 0.21 mmol) was dissolved in dry *N*,*N*-dimethylformamide (3 mL) at room temperature under nitrogen atmosphere. The tris(2-aminoethyl)-amine (8 mg, 8.19 μL, 0.055 mmol) was dissolved in dry *N*,*N*-dimethylformamide (1.0 mL) followed by dropwise addition into the reaction mixture under nitrogen atmosphere at room temperature and the reaction mixture was set aside for 24 h. Reaction was monitored by TLC. After the reaction was complete, the solvent was evaporated under reduced pressure and the residue was triturated with diethyl ether (3 × 10 mL). The crude solid product was washed with 1 N HCl (3 × 10 mL) and distilled water. The crude compound was purified through silica gel column chromatography with a dichloromethane methanol (70:30) solvent system to give pure compound [**3**] (90 mg, yield 49%). $[\alpha]^t_D$ −229.8 (*c*, 1.08 CH_3OH); R_f 0.3 in 20% MeOH−DCM; mp 97−100 °C; ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS) δ (ppm) 1.19−1.29 (m, 6H), 1.40−1.47 (m, 6H), 1.55−1.59 (m, 6H), 2.03 (t, J = 7.25 Hz, 6H), 2.40−2.43 (t, J = 6.5 Hz, 6H), 2.54 (d, J_{gem} = 12.6 Hz, 3H), 2.76−2.78 (dd, J = 5.0 Hz, J_{gem} = 12.6 Hz, 6H), 3.02−3.07 (m, 6H), 4.10 (s, 3H), 4.28 (s, 3H), 6.32 (s, 3H), 6.39 (s, 3H), 7.67 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS) δ (ppm) 25.8, 28.6, 28.8, 35.7, 37.4, 39.8, 54.0, 55.9, 59.7, 61.6, 163.3, 172.7; HRMS $[M + H]^+$ for $C_{36}H_{61}N_{10}O_6S_3$ calcd 825.3938, obsd 825.3939.

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Supporting Information Available: Experimental details, fluorescence optical micrographs, SEM images, and HRMS and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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