Integrative Self-Sorting in a Three Component System Leading to Formation of Long Fibrous Structures

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In this paper we report the integrative self-sorting processes in a three-component system consisting of β-CD, a bis-adamantane derivative AD-AD and a bis-pyromellitic diimide derivative PI-PI in a 2:1:1 ratio, leading to the slow evolution of long fibers. Using TEM, AFM and time-dependent ICD measurements of the three-component system and the constituent two-component systems (β-CD/AD-AD and β-CD/PI-PI), involvement of two social self-sorting and two narcissistic self-sorting processes, which led to the evolution of the long fibers, were identified. Initially a bis-inclusion complex of β-CD and AD-AD is formed through a social self-sorting process. The bis-inclusion complex undergoes a narcissistic self-assembly to give vesicles. The key step in the integrative self-sorting is binding of PI-PI to the vesicle surface through rim-binding association. The rim-binding motif is effectively exploited herein to perform the functions of vesicle recognition, and vesicle joining.

In 2003, Isaacs and co-workers defined self-sorting in artificial self-assembling systems as the spontaneous, high fidelity recognition of self from non-self within a mixture of similar components and classified this form of self-assembly into social self-sorting and narcissistic self-sorting.[1] Social self-sorting refers to the self-assembly of different species whereas narcissistic self-assembly refers to assembly of the same species.[2] Recently Schmittel and co-workers have outlined few guidelines for the systematic classification of self-sorted systems and even suggested a formula to evaluate the degree of self-sorting in self-assembled systems.[3,4] Self-sorting is ubiquitous in nature and manifests itself from the very simple processes such as the separation of oil/water mixture into immiscible layers to the organization of large numbers of constituents into functional assemblies such as organelle and cells.[5] During the last decade several reports dealing with self-sorting processes in synthetic systems have appeared in the literature. In most of the artificial self-sorting cases, formation of multiple assemblies in a mixture is the theme,[3d, 4a, 6] but few cases where self-sorting resulted in the formation of a single aggregate as in biological assemblies are also available.[4c–d] In this communication we report and analyze the self-assembly and integrative self-sorting processes observed in an aqueous solution containing a 2:1:1 mixture of native β-cyclodextrin (β-CD)[7], a bis-adamantane derivative AD-AD and a bis-pyromellitic diimide derivative PI-PI. We observed that an integrative self-sorting process, which involved social and narcissistic self-sortings at different stages of the self-assembly, took place in the above system, which ultimately resulted in the formation of long fibrous assemblies.

Structures of the molecules used in this study are shown in Scheme 1. AD-AD has adamantane moieties at both ends of the molecule and these are known to undergo inclusion in β-CD cavities[8] leading to the formation of bis-inclusion complex β-CD⊂AD-AD⊃β-CD as shown in Scheme 1. The β-CD⊂AD-AD⊃β-CD bis-inclusion complex can further self-assemble into stable vesicles having diameters in the range 70–500 nm.[9] Pyromellitic diimide (PI) exhibits a propensity to bind to the narrow rim of β-CD (designated as ‘rim-binding’) and hence the ditopic molecule PI-PI is expected to rim-bind with β-CD at both ends to give the bis rim-binding complex β-CD⊂β-CD⊂PI-PI⊂β-CD as shown in Scheme 1.[10] β-CD molecule also can simultaneously bind an AD moiety in the inclusion mode and a PI moiety in the rim-binding mode to give ternary complexes, such as AD-AD⊂β-CD⊂PI-PI (Scheme 1).[10b, 11] Thus, in an aqueous solution containing AD-AD, PI-PI and β-CD in a 1:1:2 ratio, one cannot apriori predict the structure of the final product, as the product formation would be governed by the strengths of various interactions described above and the preference for any sorting processes that may occur. We actually observed slow evolution of long fibers in the system during a three day period. Control experiments have shown that all the three components are incorporated into these structures. We also report that these fiber-like structures can be disassembled into smaller fragments upon addition of the competitive β-CD binder, adamantane carboxylate[12] (ADC), which confirms the non-covalent nature of the assembly.

In an aqueous solution containing β-CD, AD-AD and PI-PI in 2:1:1 ratio, formation of fibers is observed one day after mixing and the process takes nearly three days to complete. We obtained AFM and TEM images of the sample at different time intervals to study the mechanistic details of the self-assembly process. Since the rim-binding association of PI with β-CD is characterized by a negative induced circular dichroism (ICD) for PI with maximum intensity at 320 nm,[11] the self-assembly proc-
ess was also followed by monitoring the ICD signal at 320 nm. Figure 1 shows the variation of the ICD signal intensity as a function of time for three days and Figures 2a-g are the AFM and TEM images taken at the different times indicated in Figure 1.

The bis inclusion complex $\beta$-CD/AD-AD/PI-PI (2:1:1) system. The AFM images correspond to the times indicated. [PI-PI] = [AD-AD] = $4 \times 10^{-4}$ M and [β-CD] = $8 \times 10^{-4}$ M.

The bis inclusion complex $\beta$-CD/AD-AD/PI-PI does not exhibit ICD signals and hence the observed ICD signal can be attributed entirely to $\beta$-CD/PI-PI interaction. For $\beta$-CD/AD-AD interaction the association constant $K_{AD}$ obtained by isothermal titration calorimetry (ITC) was $6.4 \times 10^4$ M$^{-1}$. For $\beta$-CD/PI-PI rim-binding association $K_{PI} = 3.1 \times 10^3$ M$^{-1}$ was obtained from ITC studies (Figure S4, SI). In the presence of two equivalents of $\beta$-CD, PI-PI exhibited ICD spectrum with maximum intensity of $9.0$ mdeg at 320 nm (Figure S5, SI). For the AD-AD/PI-PI/β-CD (1:1:2) mixture at zero time, the ICD intensity observed was about $-1$ mdeg (Figure 1) suggesting that nearly $10\%$ of PI-PI are engaged by rim-binding which is slightly more than expected based on the ratio of the strengths of $\beta$-CD/AD-AD and $\beta$-CD/PI-PI interactions (as dictated by the magnitude of $K_{AD}$/$K_{PI}$). Most probably some amount of ICD active ternary complex AD-AD$\beta$-CD$\beta$-CD may also be present at zero time.

The TEM and AFM taken immediately after mixing did not show the presence of any nanostructures. TEM and AFM images taken after 5 h showed large numbers of spherical vesicular nano-structures (Figure 2a, b) which were similar to the $\beta$-CD/AD-AD/β-CD vesicles reported recently. Images obtained after 24 h showed vesicles and fibers. The AFM image clearly showed joining together of vesicles (insets in Figure 2d) suggesting that fibers are formed by fusion of vesicles. At the end of three days most of the vesicles have disappeared (Figure 2e, f). The height analysis in Figure 2f shows that vesicles and fibers have the same height, further confirming that fibers are formed from vesicles. The long fibers obtained exhibited negative ICD signal, suggesting that PI-PI is incorporated in it in the rim-binding mode.

We recently reported formation of vesicles in 2:1 $\beta$-CD/AD-AD solutions. No such self-assembly was observed in 2:1 $\beta$-CD/PI-PI solutions. We propose that the vesicles seen in Figures 2a,b are the $\beta$-CD/AD-AD/β-CD vesicles and the fibrous structures are formed by the joining together of vesicles by their interaction with PI-PI. In order to confirm this proposal PI-PI was added to pre-formed $\beta$-CD/AD-AD/β-CD vesicles. We observed formation of long fibers upon addition of 0.25 – 1.0 equivalent of PI-PI (Figure S12, SI). This reaction was also followed by ICD.

In the above experiment, the $\beta$-CD/AD-AD/β-CD vesicles are not ICD active and the ICD intensity was zero initially. One equivalent of PI-PI was added and the ICD intensity at 320 nm was monitored for 3 days. The ICD intensity vs time plot (Figure S7, SI) shows gradual growth of ICD signal and the final intensity was the same as in Figure 1. TEM and AFM images obtained after 72 h were similar to those in Figure 2e,f. This experiment suggested that PI-PI was responsible for the joining of the vesicles leading to formation of long fibres.
In another control experiment one equivalent of AD-AD was added to an aqueous solution containing β-CD and PI-PI (2:1) and the ICD intensity was monitored for 72 h. The β-CD/PI-PI solution exhibited ICD with maximum intensity of 9.0 mdeg at 320 nm (Figure S8, SI). Addition of AD-AD resulted in immediate decrease of the ICD intensity to 1.0 mdeg and from there on the variation of ICD (Figure S8, SI) was similar to that in Figure 1. Using TEM and AFM we could establish formation of vesicles and their joining together to form long fibrous structures in this case as well. These studies show that regardless of the mode of addition, the three component system of β-CD/AD-AD/PI-PI first assembles into β-CD/AD-AD/β-CD vesicles which are then joined together by PI-PI to give long fibers. Since the fibrous structures obtained integrates all three components, its formation is an example of integrative self-sorting in a three component system.

The first step (marked as step A in Scheme 2) is a social self-sorting process which involve 2:1 inclusion complexation to form β-CD/AD-AD/β-CD and 2:1 rim-binding complexation to form β-CD-PI-PI-β-CD. A small fraction of the components may also be involved in ternary complex (PI-PI-β-CD-AD-AD) formation. Both β-CD-PI-PI-β-CD and PI-PI-β-CD-AD-AD are ICD active and together constitute ~10% of the social self-sorting process. β-CD-AD-AD-β-CD formed then undergo narcissistic self-assembly to give vesicles (steps B and C). In the presence of PI-PI the vesicles join together through another social self-sorting process (step D) to give the fibrous structures. The time-dependent ICD experiments confirm that joining of the vesicles is a chemical stimuli-induced process which is brought about by the β-CD/PI rim-binding interaction.

The outer surface of the β-CD-AD-AD-β-CD vesicles are made up of the small rim of β-CD as shown in Scheme 3. The outer surface of the vesicles can recognize PI moieties and undergo rim binding with them. By the same reasoning the ditopic molecule PI-PI can also undergo rim binding with vesicles. The ditopic derivative PI-PI can complex with vesicles at both ends. When several PI-PI molecules get involved in bis rim-binding interaction with two vesicles, a mechanism for joining of vesicles as shown in Scheme 3 will be established.
We observed that pre-formed $\beta$-CD-AD-AD-$\beta$-CD vesicles react with PI and PI-PI and in both cases the reaction proceed with observation of ICD signals as shown in (Figure S9 SI). Although addition of PI led to observation of ICD signals, fiber-like structures could not be detected in this reaction. The proposal in Scheme 3 for vesicle joining is confirmed through the observation of negative ICD signals along with AFM and TEM images of fibers formed following the addition of PI-PI to pre-formed $\beta$-CD-AD-AD-$\beta$-CD vesicles. The argument is further corroborated by the observation of long fibers by TEM up on addition of 0.25 – 1.0 eq. of PI-PI to $\beta$-CD-AD-AD-$\beta$-CD vesicles (Figure S12, SI). The formation of fibers from vesicles even in the presence of 0.25 eq. of PI-PI confirmed that the fibers are formed by joining of vesicles and not by repetitive host-guest interaction between the three components.

Although all the three components are present in the required proportion at zero time, an examination of Figure 1 reveals that development of the negative ICD signal and formation of fibrous structures are initiated only around 15 h after mixing. Such a time lag is also seen in Figure S8 (SI). This lag phase, however, is not observed in Figure S7 (SI) where PI-PI was added to preformed vesicles. In control experiments AFM images of $\beta$-CD-AD-AD (2:1) system were obtained in 3 h intervals after mixing. Vesicles could be seen only after 5–6 h of mixing and these gradually grow in size till 24 h. The lag phase observed in Figures 1 and S8 suggest that joining of vesicles can take place only after the vesicles have grown to certain sizes. This is understandable because vesicle joining can occur only if large numbers of PI-PI molecules undergo rim-binding interactions at a given area on the vesicle surface.

We observed that addition of slight excess of ADC (2.25 eq w.r.t the $\beta$-CD) $\beta$-CD-AD-AD-$\beta$-CD vesicles or the $\beta$-CD-AD-AD-$\beta$-CD-PI-PI fiber solutions led to disappearance of the vesicle/fiber structures in few hours as evidenced from the absence of these structures in TEM and AFM images. In the case of long fibers, disassembly process was slow and intermediate stages could be captured by TEM and AFM (Figure S13, SI). In the case of the fibrous structures, addition of ADC led to disappearance of ICD spectrum, which further confirmed the disassembly process (Figure S13c, SI). This study confirmed the fibrous structures are formed through non-covalent interactions.

In summary, we observed integrative self-sorting in an aqueous solution containing $\beta$-CD, AD-AD and PI-PI in 2:1:1 ratio. Long fibrous structures slowly evolved from the aqueous $\beta$-CD/AD-AD/PI-PI solution over a period of three days. The fibers can also form by the addition of PI-PI to pre-formed $\beta$-CD-AD-AD-$\beta$-CD vesicles. Alternately, addition of AD-AD to pre-formed bis rim-binding complex $\beta$-CDs-PI-PI-$\beta$-CD also resulted in the formation of fibers. In this case, $\beta$-CDs-PI-PI-$\beta$-CD will disassemble (as evidenced by the immediate disappearance of ICD) and the fibers have to evolve through all the stages shown in Scheme 2. All the evidences suggested that the fibers are formed through the joining of vesicles induced by the ditopic rim-binding interaction of PI-PI. This study shows that the rim-binding motif can be effectively exploited to perform the functions of vesicle recognition and joining leading to formation of larger structures. In principle, the rim-binding motif invoked here for vesicle recognition and joining is applicable to all native $\beta$-CD based vesicles because the outer surfaces of these vesicles are comprised of the narrow rim of $\beta$-CD. Studies to verify this hypothesis are in progress in our laboratory.

**Supporting Information**

Electronic supporting information (ESI) is provided for this article which contains synthesis and detailed characterization of PI-PI (NMR and HRMS spectra), isothermal titration calorimetry (ITC), $^{1}H$ NMR titration and time dependent Induced Circular Dichroism (ICD) spectra. This also contains additional AFM and TEM images of nanofibers formed by vesicle fusion and their guest induced disassembly.

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