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Molecular "Chaperones" Guide the Spontaneous Formation of a 15-Component Hydrogen-Bonded Assembly

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The formation of synthetic hydrogen-bonded structures is typically a fast and reversible process that occurs instantaneously upon mixing the individual components in solution.[1] In the majority of cases, the energy of interaction between the individual components is sufficiently low for the assembly process to be fully under thermodynamic control. Therefore, product formation is usually quantitative, because initially misformed structures can dissociate and recombine to give the correct assembly. Whereas the formation of kinetically inert metal-coordination complexes is well-known,[2] examples of kinetically stable hydrogen-bonded assemblies have hardly been reported.[3] However, it is expected that a further increase in the number of individual interactions will inevitably lead to kinetic control in hydrogen-bond directed self-assembly processes and seriously limit the potential for the noncovalent synthesis of H-bonded structures of ≥ 3 nm.

This seemingly unsolvable problem has long ago been addressed by Nature, as part of the folding process of biologically active proteins. Here the use of so-called "chaperones", which are small protein-like molecules that assist in the folding process by stabilizing metastable conformations of the protein on the way to its bioactive conformation, provides a solution to the problem. To the best of our knowledge the use of "chaperones" to direct the formation of synthetic noncovalent assemblies has thus far not been reported. In this communication, we describe an example of small molecules acting as molecular "chaperones" in the formation of synthetic noncovalent assemblies $\mathbf{2}_3$·$(BuCYA)_{12}$. In the absence of the chaperones, formation of nondefined structures is observed, which are kinetically inert and cannot rearrange to give assembly $\mathbf{2}_3$·$(BuCYA)_{12}$ (Figure 1).

The self-assembly of multicomponent rosette assemblies, like $\mathbf{1}_3$·$(DEB)_6$ and $\mathbf{2}_3$·$(DEB)_{12}$, is a fast and reversible process that occurs within seconds after mixing the components together at ambient temperature.[4] ^{1}H NMR spectroscopy studies have shown that the formation of assembly $\mathbf{2}_3$·$(DEB)_{12}$ is quantitative (Figure 2a) and that exchange of the individual components rapidly takes place at room temperature. However, assembly experiments of **2** with BuCYA (*N*-butyl cyanuric acid) instead of DEB (5,5-diethyl barbituric acid) gave entirely different results than were expected. For example, it was found that mixing of tetramelamine **2a** or (*R*,*R*)-**2b** with BuCYA (1:4 ratio) in chloroform did not show the expected formation of the corresponding assembly $\mathbf{2}_3$·$(BuCYA)_{12}$. Instead, the ^{1}H NMR spectra show a complicated set of signals (Figure 2b), suggesting that a mixture of different assemblies had been formed.[5] These nondefined structures display an extremely high kinetic stability. Formation of assembly $\mathbf{2a}_3$·$(BuCYA)_{12}$ was never observed, not even after heating the mixture for 2 weeks at reflux, whereas tetrarosette $\mathbf{2b}_3$·$(BuCYA)_{12}$ was finally formed after heating the solution in chloroform overnight.

Analysis of the 1:4 mixture of **2a** and BuCYA by gel permeation chromatography (GPC) showed the elution of structures with a broad molecular weight (MW) distribution. Analysis of the mixture of **2b** with BuCYA by CD spectroscopy strongly suggests that the ill-defined structures contain double rosette subdomains, regarding the close resemblance with the CD-spectra of the tetrarosette assemblies $\mathbf{2b}_3$·$(DEB)_{12}$ and $\mathbf{2b}_3$·$(BuCYA)_{12}$. The assemblies are strongly CD-active as a result of the exclusive formation of *M*-helices as induced by the chiral centers in (*R*,*R*)-**2b**.[6]

To our surprise, we found that the spontaneous formation of assembly $\mathbf{2a}_3$·$(BuCYA)_{12}$ and $\mathbf{2b}_3$·$(BuCYA)_{12}$ occurs cleanly and without any byproducts upon mixing either tetramelamines **2a** or **2b** in the presence of DEB (4.0 equiv) with BuCYA (1:4 ratio) at room temperature in $CDCl_3$. The ^{1}H NMR spectra of both mixtures (Figure 2c) clearly show the expected signals for the cyanurate $NH_{a,b}$ and melamine $NH_{c,d}$ proton signals, together with an intense signal for free DEB around 8.4 ppm. Heating the solution of assembly $\mathbf{2a}_3$·$(BuCYA)_{12}$ in $CDCl_3$ for 2 weeks did not change the ^{1}H NMR spectrum. Furthermore, after heating of the undefined assemblies obtained upon mixing of tetramelamine **2a** and BuCYA for 2 days in toluene-d_8 at 100 °C, the ^{1}H NMR spectrum showed the four characteristic peaks for the hydrogen-bonded cyanurate $NH_{a,b}$ of tetrarosette assembly $\mathbf{2a}_3$·$(BuCYA)_{12}$, indicating that it is indeed the thermodynamically most stable structure. GPC-analysis of the tetrarosette assembly $\mathbf{2a}_3$·$(BuCYA)_{12}$ obtained under the influence of DEB clearly shows that this hydrogen-bonded structure has a well-defined composition, clearly different from that obtained upon directly mixing **2a** with BuCYA. Also the CD-spectrum of $\mathbf{2b}_3$·$(BuCYA)_{12}$ is quite different in comparison to that of the 1:4 mixture of **2b** and BuCYA. The positive CD between 270 and 330 nm has clearly shifted to lower wavelength, much more closely resembling the CD of the corresponding tetrarosette $\mathbf{2b}_3$·$(DEB)_{12}$.

To further establish the assistance of DEB in the formation of tetrarosette assembly $\mathbf{2}_3$·$(BuCYA)_{12}$, several control experiments were performed. First of all, it was established that DEB exclusively is active in conjunction with *free* tetramelamine **2a**. Addition of DEB to **2a** that has already been exposed to BuCYA did not lead to the observed chaperone effect. Similarly, mixing of free **2a** with a mixture of BuCYA and DEB (ratio 1:1) did not lead to formation of assembly $\mathbf{2a}_3$·$(BuCYA)_{12}$.

Subsequently, we studied the influence of weak Brønsted acids with pK_a values slightly higher or lower than DEB (pK_a = 7.97),[7] like 4-nitrophenol (pK_a = 7.15), 3-bromophenol (pK_a = 8.85), and

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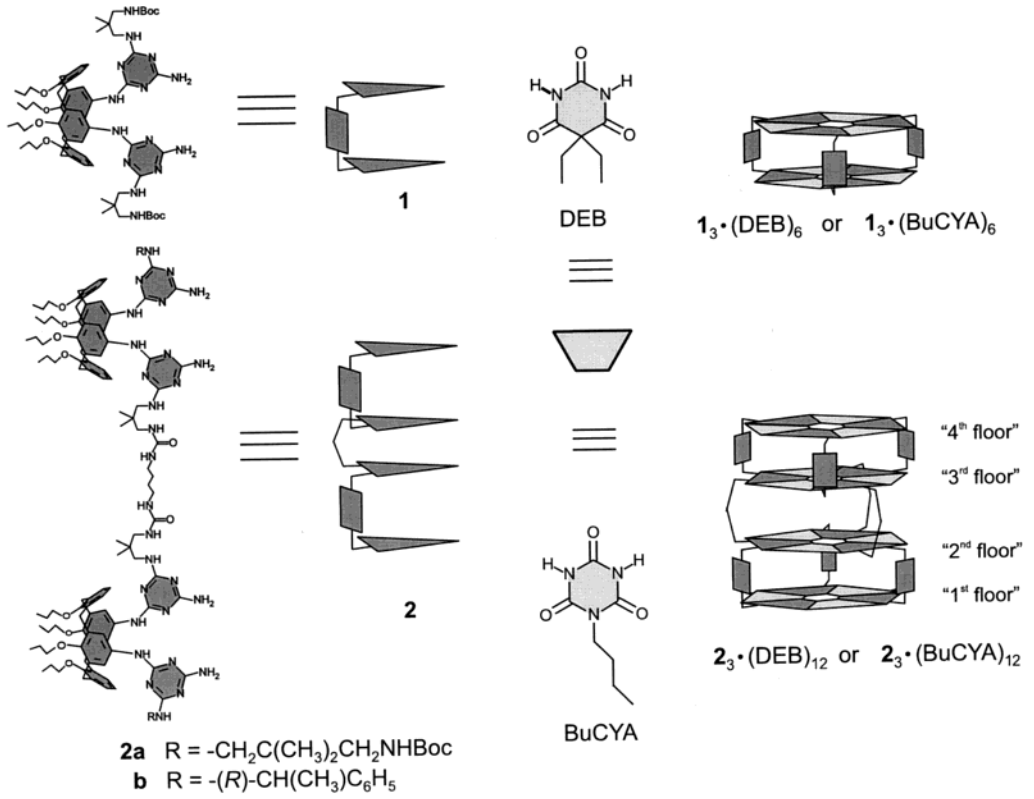

Figure 1. Schematic representation of double [$\mathbf{1}_3$·(DEB)$_6$ or $\mathbf{1}_3$·(BuCYA)$_6$] and tetrarosette [$\mathbf{2}_3$·(DEB)$_{12}$ or $\mathbf{2}_3$·(BuCYA)$_{12}$] assemblies, together with the molecular structure for the individual components.

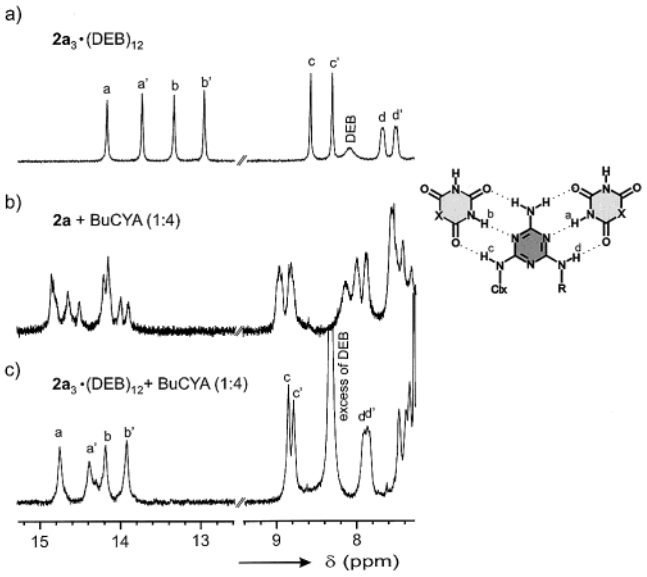

Figure 2. ^{1}H NMR spectra (300 MHz) of (a) $\mathbf{2a}_3$·(DEB)$_{12}$, (b) 1:4 mixture of **2a** and BuCYA immediately after mixing, (c) $\mathbf{2a}_3$·(BuCYA)$_{12}$ obtained via treatment of $\mathbf{2a}_3$·(DEB)$_{12}$ with 4 equiv of BuCYA. Spectra in $CDCl_3$ at 293 K. (NH_{a-d}, first and fourth floors and $H_{a'-d'}$, second and third floors of tetrarosette, Figure 1).

2,4-dinitrophenol ($pK_a = 3.96$). None of these compounds was able to exert a similar effect when added to **2a** prior to assembly formation,[8] which excludes the possibility that the DEB units act as an acid catalyst. Addition of 4 equiv of phthalimide, which contains only one ADA H-bonding motif, to **2a** did not favor the formation of tetrarosette assembly $\mathbf{2a}_3$·(BuCYA)$_{12}$ either. Apparently, the presence of both ADA H-bonding motifs as present in DEB is a prerequisite for the observed effect.

In conclusion the results described in this communication provide an unprecedented example of small molecules acting as chaperones in the spontaneous assembly of noncovalent structures and thus provides a novel principle that potentially widens the scope of noncovalent synthesis.

Supporting Information Available: GPC data of **2a** and $\mathbf{2a}_3$·(BuCYA)$_{12}$ and CD data of **2b** and $\mathbf{2a,b}_3$·(BuCYA)$_{12}$ (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Prins, L. J.; Timmerman, P.; Reinhoudt, D. N. *Angew. Chem., Int. Ed.* **2001**, *40*, 2382−2426.
(2) (a) Cardenas, D. J.; Sauvage, J. P. *Inorg. Chem.* **1997**, *36*, 2777−2783. (b) Ibukuro, F.; Kusukawa, T.; Fujita, M. *J. Am. Chem. Soc.* **1998**, *120*, 8561−8562. (c) Hasenknopf, B.; Lehn, J.-M.; Boumediene, N.; Leize, E.; Van Dorsselaer, A. *Angew. Chem., Int. Ed.* **1998**, *37*, 3265−3268.
(3) (a) Mathias, J. P.; Simanek, E. E.; Seto, C. T.; Whitesides, G. M., *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1766−1769. (b) Prins, L. J.; De Jong, F.; Timmerman, P.; Reinhoudt, D. N. *Nature (London)* **2000**, *408*, 181−184.
(4) Jolliffe, K. A.; Timmerman, P.; Reinhoudt, D. N. *Angew. Chem., Int. Ed.* **1999**, *38*, 933−937.
(5) Mixing **1** and 2.0 equiv. of BuCYA in $CDCl_3$ gives only $\mathbf{1}_3$·(BuCYA)$_6$, formed upon gently heating (<1 min).
(6) Prins, L. J.; Huskens, J.; De Jong, F.; Timmerman, P.; Reinhoudt, D. N. *Nature (London)* **1999**, *398*, 498−502.
(7) Mascal, M.; Fallon, P. S.; Batsanov, A. S.; Heywood, B. R.; Champ, S.; Colclough, M. *J. Chem. Soc., Chem. Commun.* **1995**, 805−806.
(8) For 4-nitrophenol the thermodynamic equilibrium was reached after extensively heating. The observed effect is much weaker than with DEB and most likely originates from its complexation inside the tetrarosette (complexation will be described elsewhere).

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