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Sequence-Specific Association in Aqueous Media by Integrating Hydrogen Bonding and Dynamic Covalent Interactions

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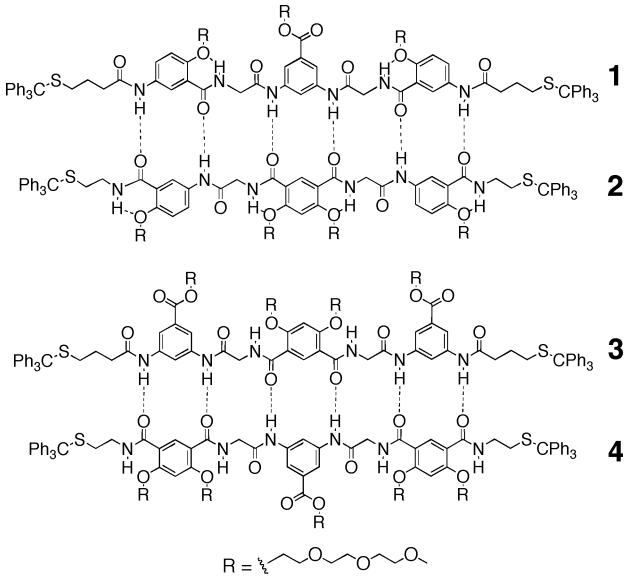
Strategies for controlling intermolecular association will lead to the creation of a variety of novel self-assembled architectures, most of which would otherwise be difficult to obtain based on covalent synthesis.[1] Due to its strength and directionality, hydrogen bonding has attracted intense interest in the design of self-assembling structures.[2] H-bonded complexes consisting of components carrying arrays (sequences) of H-bond donors and acceptors have been used as associating units for directing intermolecular interactions.[3] Highly specific H-bonded units have been applied to the creation of supramolecular constructs, such as supramolecular polymers,[4] dendrimers,[5] and liquid crystal materials.[6] In recent years, we reported H-bonded duplexes based on the zippering of oligoamide strands bearing complementary H-bonding sequences.[7] The duplexes we developed are featured by programmable sequence specificity and tunable stability. These duplexes have been used as sequence-specific associating units for the nucleation of β-sheet structures when attached to natural oligopeptides,[8] for the templation and direction of chemical reactions,[9] and for the noncovalent ligation of strongly phase-separating polymer blocks.[10] Like most other H-bonded complexes, our H-bonded duplexes are stable only in nonpolar solvents. Their dissociation in aqueous media hampers applications involving biological conditions. Although several systems with microenvironments that promote H-bonding in aqueous media have been reported,[11] in competitive environments, the association of relatively small molecular components based on H-bonding is still a largely unsolved fundamental problem. In contrast, in nature, nearly all water-soluble self-assembling systems are formed based on the cooperative interaction of multiple noncovalent forces. For example, the extensively studied DNA duplexes are stabilized by both H-bonding and aromatic stacking interactions.

A possible strategy for achieving specific intermolecular association in polar media is to combine H-bonding with other forces. In this paper, we would like to report the sequence-specific association of molecular strands into covalently cross-linked duplexes in aqueous media based on the interplay of H-bonding and dynamic covalent[12] interactions.

Oligoamides **1**, **2**, **3**, and **4** were designed. In nonpolar media, strands **1** and **2**, and similarly, **3** and **4**, can sequence-specifically associate into highly stable, six-H-bonded duplexes **1·2** and **3·4**.[7b,d] It is known that, under redox conditions, *S*-trityl groups can lead to the reversible formation of disulfide bonds.[13] The formation of disulfide bonds between the oligoamide strands should result in their covalent cross-linking. In this system, the reversibility in the formation of disulfide bonds is crucial to the retention of the H-bonding sequence specificity. The fully matched H-bonding sequences between **1** and **2** and between **3** and **4**, along with the presence of two disulfide linkages in each pair, should lead to thermodynamically stable, disulfide cross-linked duplex products **1**−**2** and **3**−**4**.[14] Other combinations with mismatched sites, such as the self-dimers of the individual oligoamide strands, should be corrected due to the reversible nature of both the noncovalent and covalent interactions.



Amides **1**, **2**, **3**, and **4** were synthesized based on the same procedures we reported previously[7−10] and were fully characterized by NMR and mass spectrometry.[14] The 1:1 mixture of **1** and **2** (0.5 mM each) was treated with iodine (6 mM) in methylene chloride, methanol, or water containing 10% THF. The reaction mixture was then examined using MALDI-TOF. In CH_2Cl_2, the disulfide cross-linked **1**−**2** was the overwhelmingly major product.[14] This is not a surprising result since in CH_2Cl_2 **1** and **2** can form a highly stable, six-H-bonded duplex in which the *S*-trityl groups are placed in close proximity. In methanol, the effect of H-bonding on the association of **1** and **2** is less clear, given that **1** and **2** cannot stably associate in polar solvents.[3b] It is interesting that, despite the high polarity of methanol, the sequence-specifically paired **1**−**2** still appeared as the dominant product.[14] To probe whether the same sequence-specific cross-linking can be achieved in the highly competitive aqueous solution, strands **1** and **2** were mixed in water (with 10% THF) in the presence of iodine.[14] In this case, MALDI indicated that **1**−**2** again appeared as the overwhelmingly major product (Figure 1a). That the formation of **1**−**2** was indeed sequence-dependent was also supported by the results obtained when **1**, **2**, or **3** alone, or the 1:1 mixture of **1** and **3**, or **2** and **3**, was treated under the same redox conditions. Without its complementary strand, **1** or **2** self-cyclized into the monomeric **1′** or **2′** via the formation of a disulfide bond. Neither **1** nor **2** paired with **3** to form the corresponding cross-linked duplex.[14]

To further verify the sequence dependence of the cross-linked products in aqueous media, a mixture containing 1 equiv (0.5 mM) of each of **1**, **2**, and **3** was treated with iodine and then examined

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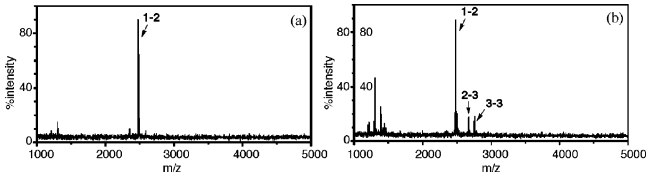

Figure 1. MALDI-TOF MS spectra of (a) the 1:1 mixture of **1** and **2** and (b) the 1:1:1 mixture of **1**, **2**, and **3**, in H_2O/THF (9/1, v/v) in the presence of iodine (6 mM).

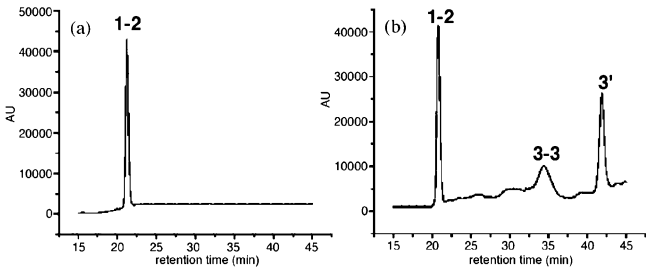

Figure 2. HPLC traces of aliquots of the solutions of (a) the 1:1 mixture of **1** and **2** and (b) the 1:1:1 mixture of **1**, **2**, and **3**, in H_2O/THF (9/1, v/v) in the presence of iodine (6 mM). Retention time: **1**−**2** (21 min); **3**−**3** (35 min); **3′** (42 min).

using MALDI-TOF. It was found that, although having four and three matched sites with **1** and **2**, respectively, strand **3** did not interfere with the formation of **1**−**2**. The cross-linked **1**−**2** was detected as the major product (Figure 1b). Compared to **1**−**2**, other possible products, such as the cross-linked **3**−**1** or **3**−**2**, did not appear in significant portion. In contrast, when mixed in water in the presence of iodine, amide **3** did pair with **4** and form the cross-linked **3**−**4** as the dominant product,[14] suggesting that the observed specific pairing and cross-linking were not a special case associated with **1** and **2** only.

While MALDI-TOF experiments provide clear, yet qualitative, evidence for the sequence-specific formation of the cross-linked duplexes in aqueous solution, the cross-linking of **1** and **2** in water was also examined by reverse phase HPLC. As shown in Figure 2a, the 1:1 mixture of **1** and **2**, after being treated with iodine in water, formed the cross-linked **1**−**2** exclusively. In contrast, strands **1** or **2** alone formed mostly the disulfide cyclized monomeric **1′** and **2′**.[14] The presence of strand **3** did not interfere with the exclusive formation of **1**−**2** (Figure 2b). The identity of each HPLC fraction was confirmed by comparing with standards or by ESI and MALDI. These results are fully consistent with those from the MALDI experiments, which provide conclusive evidence for the sequence-dependent nature of the formation of the disulfide cross-linked duplexes in aqueous media.

The efficiency of the sequence-dependent disulfide cross-linking was also demonstrated by isolating **1**−**2** from a reaction on a larger scale.[14] The cross-linked crude product **1**−**2** formed nearly quantitatively and was still obtained in 80% yield even after exhaustive purification using flash column chromatography followed by reverse phase HPLC.

The above results clearly indicate that, in highly competitive media, such as methanol and water, by introducing reversible covalent forces into a H-bonded system, the high sequence specificity offered by the H-bond arrays can be completely preserved. This discovery is significant in several aspects: (1) The combination of the sequence specificity of H-bond arrays and the strength of disulfide bonds offers a novel class of molecular associating and ligating units that are applicable in both polar and nonpolar solvents. (2) The programmable specificity of our H-bonded duplexes allows the generation of a large number of duplexes that are cross-linked sequence-specifically by disulfide bonds, which greatly expands the diversity of this important, but symmetrical, dynamic covalent interaction. Using these supramolecular−dynamic covalent linking units, the directed, specific formation of covalently linked structures and materials based on a self-assembling fashion under mild, biocompatible conditions can be envisioned. (3) Fundamentally, this system offers a platform for studying the role of H-bonding interaction in water and other competitive media. Obviously, H-bonding interactions play a critical role in this case. Such a role can be either kinetic, based on the sequence-specific association of the complementary stands, which increases the effective molarity of the disulfide formation reaction, or thermodynamic, based on the stabilization of the cross-linked products through fully matched H-bonding sequences. Elucidating the corresponding mechanism should lead to a deeper understanding and better control of H-bonding in aqueous media, a challenge worthy of addressing.

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Supporting Information Available: Experimental details, NMR data, MS spectra, HPLC traces, and structures of cross-linked products. This material is available free of charge via the Internet at http://pubs.acs.org.

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