Dynamic Nanostructures

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Constitutional Adaptation of Dynamic Polymers: Hydrophobically Driven Sequence Selection in Dynamic Covalent Polyacylhydrazones**

*J. Frantz Folmer-Andersen and Jean-Marie Lehn**

Dedicated to Professor Helmut Ringsdorf on the occasion of his 80th birthday

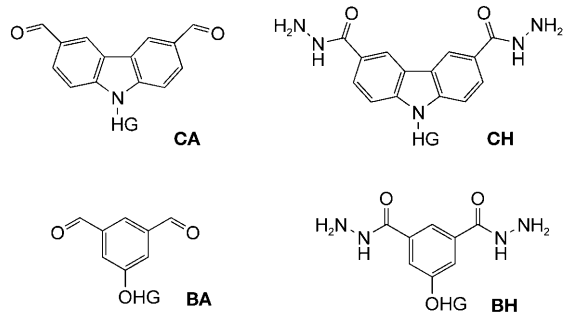
The folding and assembly of linear biological macromolecules into specific supramolecular architectures on the basis of their primary sequences embodies the flow of structural information from the molecular level to larger scales through the iterative application of reversible (dynamic) supramolecular forces. Understanding and controlling such up-scale propagation of structural information offers the potential to impose precise order (and attendant function) at the nano- and mesoscopic levels.

The processing of structural information by interactional algorithms[1] is a central theme of constitutional dynamic chemistry (CDC),[2] which embodies the use of reversible covalent or noncovalent interconnections to build up higher-order dynamic entities of molecular or supramolecular nature from molecular subunits. In recent years, the implementation of CDC in polymer science has led to the development of dynamic polymers, or dynamers, which are generated from monomers joined by either supramolecular or reversible covalent interconnections.[3] By virtue of their capacity to undergo monomer incorporation, exchange, and reorganization processes, dynamers are especially attractive as stimulus-responsive materials.[4] Variation in dynamer constitution through component selection has been shown to occur in response to various external triggers,[5] as well as in self-organization-driven selection in gel formation.[6]

A less developed aspect of dynamer chemistry is the possibility it offers of interrogating structural relationships within macromolecules. In the strictly hierarchical folding of conventional, constitutionally static polymers, a fixed monomer sequence determines the folded structure. In contrast, an appropriately designed dynamer may exhibit an interdependence between primary and secondary structure, through which sequences that adopt well-defined folded structures may spontaneously assemble by selection of the appropriate components under the pressure of folding stabilization as an internal driving force. This scenario is particularly intriguing in view of postulates that protein folding (analog information) has driven the adaptation of primary sequences (digital information) on an evolutionary timescale.[7] The prospect of using folding energies to select for the expression of certain dynamer sequences is also highly attractive from the standpoints of nanosynthesis and materials chemistry.

In pursuit of folding-driven sequence selection, we sought to develop dynamers with well-defined secondary structures. Previous studies have established that folding can enhance the stabilities of reversibly linked macromolecules while giving rise to nucleation–growth (N–G) behavior.[3c,8] In view of the importance of hydrophobicity in biomacromolecular folding, we designed amphiphilic dialdehyde (DA; **CA** and **BA**) and di(acylhydrazine) (DH; **CH** and **BH**) monomers[9] consisting of a lipophilic core (a substituted carbazole (**C**) or benzene (**B**)) and a hydrophilic hexaglyme (HG) chain (Scheme 1). The reversible, acid-catalyzed polycondensation of a given DA/DH pair would be expected to generate a polyacylhydrazone dynamer,[3b,d] presenting a nonpolar main chain from which hydrophilic HG moieties emanate at regular intervals. It was envisioned that such local amphiphilicity would promote the hydrophobically driven formation of recurrent folded structures, which may preferentially accommodate certain monomer sequences on the basis of both their structural fit and their interactions within the folded superstructure.

The four possible polymers poly(**CA**-**CH**), poly(**BA**-**BH**), poly(**CA**-**BH**), and poly(**BA**-**CH**) were synthesized by acid-



Scheme 1. Structures of dialdehyde (DA; **CA** and **BA**) and dihydrazide (DH; **CH** and **BH**) monomer subunits used in this study. HG = $(CH_2CH_2O)_6CH_3$.

[*] Dr. J. F. Folmer-Andersen,[+] Prof. J.-M. Lehn
Institut de Science et d'Ingénierie Supramoléculaires (ISIS)
Université de Strasbourg
8 allée Gaspard Monge, 67000 Strasbourg (France)
Fax: (+33) 3-9024-5140
E-mail: lehn@isis.u-strasbg.fr
Homepage: http://www-isis.u-strasbg.fr/supra/

[+] Present address: Department of Chemistry
The State University of New York at New Paltz
75 S. Manheim Blvd, New Paltz, NY 12561 (USA)

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catalyzed polycondensation and characterized by methods including small-angle neutron scattering (SANS) and transmission electron microscopy (TEM). Consistent with previous studies of structurally related glycodynamers,[3d] rodlike nanostructures were observed in solution. Fitting of the SANS profiles provides estimates of averaged molecular weights and the structural dimensions of the rods, which are relatively consistent throughout the series (Table 1). Imaging by TEM allows for visualization of the rods, together with lateral aggregates (Figure 1a,b), and verifies their physical dimensions. The constant diameters (ca. 4.5 nm) and variable lengths of the filaments, along with the absence of branching, imply that each corresponds to an individual polymer organized into a regular folded structure. The observed morphology is inconsistent with those expected of extended polymer chains or random coils but can be well accounted for on the basis of a tubular helix model, in which the aromatic main chain undergoes hydrophobic π stacking, while the HG groups are exposed (Figure 1c).

Table 1: Physical dimensions of rodlike polymers as determined by fitting of SANS data.[a]

Sample	M_w [kDa]	R_g [nm]	μ [kDa nm^{-1}]	d_c [nm]
poly(**BA-CH**)	340 ± 40	11 ± 2	8.4 ± 10	4.4 ± 0.3
poly(**CA-CH**)	280 ± 30	10 ± 2	7.5 ± 9	4.4 ± 0.3
poly(**BA-BH**)	140 ± 20	6 ± 2	6.7 ± 8	4.1 ± 0.3
poly(**CA-BH**)	200 ± 20	8 ± 2	6.6 ± 8	4.4 ± 0.3

[a] M_w = weight-averaged molecular weight, R_g = radius of gyration, μ = linear mass density, d_c = cross-sectional diameter.

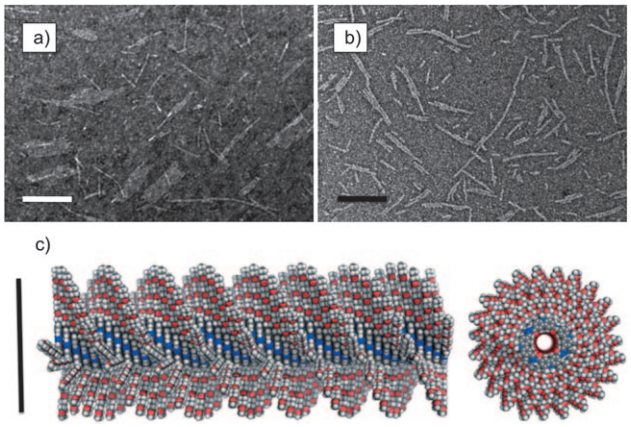

Figure 1. TEM images demonstrating rodlike morphologies of a) poly(**CA-CH**) and b) poly(**CA-BH**), prepared by mixing the appropriate monomers (6 mM each) at pH 1.7 and 20 °C for 14 d. Scale bars = 100 nm. c) Molecular model of a poly(**CA-CH**) segment constructed from SANS data, molecular modeling, and transmission electron micrographs as viewed from the side and along the rod axis; the helical pitch and linear mass density are 3.42 Å and 6.4 kDa nm^{-1}, respectively. Scale bar = 6 nm.

Reversibly formed polymers with well-defined secondary structures can exhibit N–G behavior.[3c,8] According to this model, stabilizing interactions between non-adjacent monomers make the addition of a monomer to the end of the chain more exergonic than the dimerization of two monomers. Under conditions of imbalanced stoichiometry, N–G can cause the suppression of oligomers and the amplification of large polymers and monomers.[3c,8b,d] Such behavior is indicated in the present polymers by the fact that they resist the incorporation of excess DH monomers. The effect is easily monitored by ^{1}H NMR spectroscopy, as in D_2O the aromatic resonances for the polymers are sufficiently broadened that they can be subtracted from the baseline, thus allowing for the selective observation of free monomers. For example, a solution initially prepared at 5 mM **CA** and 10 mM **BH** contains polymeric poly(**CA-BH**) and nearly 5 mM unreacted **BH** at equilibrium, according to quantitative NMR spectroscopy and size-exclusion chromatography measurements.[9] This result contrasts the distribution of short oligomers expected of a statistical polymerization with a DH:DA ratio of 2:1. The demonstrated N–G behavior not only provides further support for well-defined folded structures but also facilitates the monitoring of monomer exchange processes by NMR spectroscopy.

Competitive polymerizations were performed in which **CA** was allowed to react simultaneously with both DH monomers in a 1:1:1 ratio. Initially, the components were mixed at pD 7.8, at which acylhydrazone condensation and exchange processes are negligible, giving a ^{1}H NMR spectrum corresponding to unreacted monomers (Figure 2a, bottom trace). The pD value was then lowered to 2.0, initiating polymerization and immediately causing the disappearance of signals for the limiting reagent **CA**, along with the suppression of signals for the DH monomers. The sample was then equilibrated at 55 °C for 12 h, over which time the aromatic resonances for **CH** (a singlet at $\delta \approx 8.5$ ppm and two doublets at $\delta = 7.9$ and 7.6 ppm) disappeared almost entirely, thus

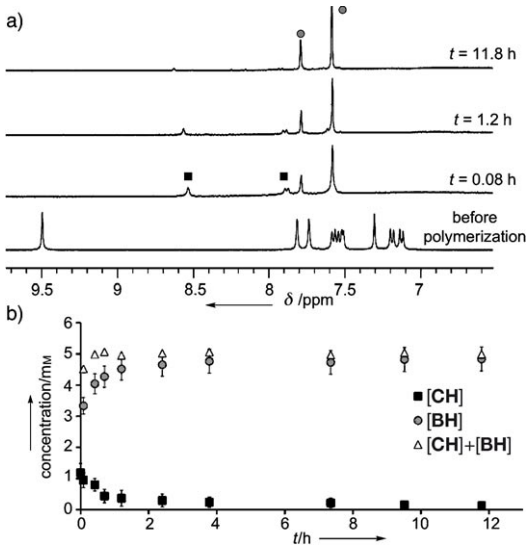

Figure 2. a) Part of the 400 MHz ^{1}H NMR spectrum of a solution of **CA** (5.1 mM), **CH** (5.0 mM), and **BH** (5.0 mM) in D_2O (5 mM phosphate buffer, pD 7.8) prior to and at various times after the adjustment to pD 2.0 with DCl and heating to 55 °C. By virtue of the monomers' amphiphilicity, their ^{1}H NMR spectral signals undergo diamagnetic shifting with increasing concentration. Selected signals arising from **CH** and **BH** are marked with black squares and gray circles, respectively. b) Monomer concentrations over the course of the competitive polymerization experiment as determined by comparison of the integrated signal intensities to that of an internal standard (*t*BuOH).

indicating its inclusion into the polymer, while those of **BH** (two singlets in 2:1 ratio at $\delta = 7.6$ and 7.8 ppm) persisted. After 12 h, **CH** was barely detectable, while the concentration of **BH** was close to the original value. A comparison of the integrated signal intensities indicates that **CH** is polymerized in preference to **BH** by a factor of at least 20:1, and that poly(**CA**-**CH**) and **BH** are the major products at equilibrium. No further changes in the spectrum were observed after one week.[10] Figure 2b plots the concentrations determined for each DH monomer during the approach to equilibrium. Immediately after polymerization, [**BH**] diminishes slightly before reemerging and leveling off, whereas [**CH**] decays steadily. This kinetic effect can be explained in terms of an initial unselective polycondensation, which is corrected by slower isodesmic acylhydrazine exchange reactions, ultimately leading to the replacement of **BH** incorporated into the polymer molecules by **CH**. Analogous experiments using **BA** instead of **CA** showed a similar selectivity for **CH** over **BH**, although the magnitude of the effect was less pronounced.

The selection for **CH**-containing sequences could also be observed in the substitution of DH monomers within preformed polymers. As shown in Figure 3, the addition of one equivalent of **CH** to poly(**CA**-**BH**) at pD 1.8 caused the near complete disappearance of the **CH** signals in the ^{1}H NMR spectrum and the concomitant emergence of those of **BH** over a period of several days. The total concentration of free DH monomer remained fixed over the course of the exchange, thus indicating that **CH** was substituted for **BH** within the polymer on approximately a 1:1 basis. No appreciable incorporation of **BH** into preformed poly(**CA**-**CH**) was observed, thus confirming that equilibrium had been established. Analogous results were obtained using **BA** as the DA component, although, as with the competitive polymerization experiments, the selectivity was lower.[9] Monomer exchange was not observed at neutral or basic conditions, even after three months, thus indicating that acylhydrazone bonds are constitutionally static under such conditions and that formation and exchange processes can be effectively controlled by changes in the pH value.

To investigate the origin of the observed selectivity, **BH** and **CH** were allowed to compete for two equivalents of benzaldehyde-2,4-disulfonic acid disodium salt (BDS) under dynamic exchange conditions. A preference for acylhydrazone formation with **CH** over **BH** would indicate that selectivity in the polymerization experiments was biased as a result of a greater intrinsic reactivity of **CH**. Such an effect was not observed, however, as a roughly equal mixture of the two acylhydrazone adducts **CH**-BDS and **CH**-BDS was produced at equilibrium.[9] This lack of selectivity indicates that factors involving the macromolecular architecture underlie the preference for **CH** in the polymeric systems. In light of the aforementioned structural characterizations, it can be reasonably assumed that the present polymers undergo hydrophobically driven folding in solution. The preferential polymerization of **CH**, which exhibits about 75 Å^2 more nonpolar surface area than **BH**, can therefore be rationalized on the basis of its greater tendency to participate in hydrophobic interactions. Additionally, the different linkage geometries of the two DH monomers might also lead to somewhat more efficient folding of **CH**-containing polymers, thus contributing to their amplification at equilibrium.

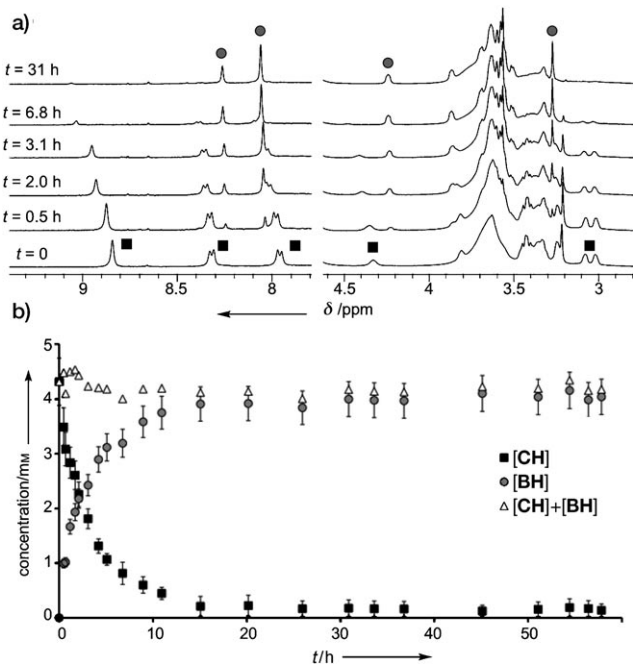

Figure 3. a) Part of the 400 MHz ^{1}H NMR spectrum of a solution of poly(**CA**-**BH**) and **CH** (4.5 mM) in D_2O (5 mM phosphate buffer, pD 7.5, 55 °C) prior to ($t = 0$) and at various times after adjustment to pD 1.8 with DCl. The original poly(**CA**-**BH**) solution was prepared by mixing **CA** and **BH** (4.5 mM each at pD 1.8 and 20 °C) for 48 h with subsequent adjustment of the pD value with NaOD. Selected signals arising from **CH** and **BH** are marked with black squares and gray circles, respectively. b) Observable monomer concentrations over the course of the exchange as determined by comparison of the integrated signal intensities to that of an internal standard (*t*BuOH). The exchange half-life is estimated to be 1.7 h.

The role of hydrophobicity in the preferential polymerization of **CH** over **BH** was evaluated by conducting competitive polymerization experiments in aqueous/organic solvent mixtures. Organic solvents are expected to act as denaturants by weakening hydrophobic interactions, and the resultant loss of secondary structure should lead to diminished sequence selectivity in a folding-driven system. Strongly denaturing solvents such as [D_7]DMF and [D_6]DMSO were found to be inappropriate in this context, as they gave rise to very complicated ^{1}H NMR spectra containing signals for polymeric and monomeric species.[11] It was found, however, that the monomers could be selectively observed in aqueous solutions containing up to 90% [D_3]MeCN, and that the presence of [D_3]MeCN elicited the anticipated loss of selectivity. Figure 4 shows a plot of equilibrium [**BH**]/[**CH**] ratios for competitive polymerizations using both DA components at various [D_3]MeCN/D_2O ratios. A clear loss of selectivity with increasing volume fraction of [D_3]MeCN is apparent in both cases, with roughly statistical mixtures of **CH** and **BH** present in the free monomer population at the highest fraction studied, implying a near complete randomization of the polymeric material.

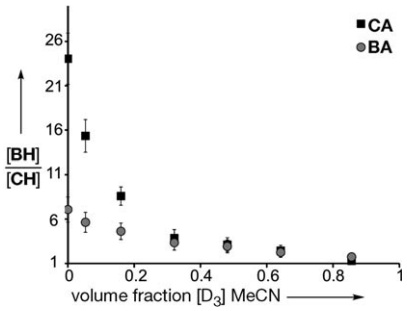

Figure 4. Equilibrium monomer ratios determined from integrated ^{1}H NMR spectral signal intensities of solutions initially containing either **CA** or **BA** (5.0 mM) and both **CH** and **BH** (5.0 mM each) at various D_2O/[D_3]MeCN (v:v) compositions. All solutions contained 5.5 mM phosphate and 10.4 mM DCl and were equilibrated for 14 h at 55 °C.

The loss of sequence selectivity on addition of [D_3]MeCN may be related to a lessening of hydrophobic effects in the mixed aqueous/organic medium. However, no significant hypochromic changes[12] were observed in the UV absorption spectra of the polymers upon transfer from MeCN to water (whereas large effects are observed with the transfer from DMF to water). Along with the persistence of N–G behavior as well as signal broadening of the ^{1}H NMR spectrum in [D_3]MeCN, this result suggests that the general polymeric superstructure is maintained at high MeCN content. In view of the sensitivity of equilibrium ratios to small energetic changes (a selectivity factor of 25:1 corresponds to an energetic factor of less than 2.1 kcal mol^{-1} at 55 °C), the observed dependence of polymer sequence on solvent composition can be explained in terms of modest variations in the folded polymer structure or changes in the solvation energies of the free monomers. Together, the selective polymerization of **CA** with **CH** versus **BH** and the strong dependence of this selectivity on solvent composition exemplify a powerful aspect of CDC, namely that supramolecular interactions (folding and solvation) can exert subtle control over dynamic covalent processes to the extent that structurally similar subunits can be efficiently discriminated. The demonstrated use of noncovalent interactions to direct constitutional variation in the covalent assembly/disassembly of nanostructured entities represents a promising strategy to selectively generate organized structures and materials of specific composition across length scales.

In a perspective of broad significance, the behavior of the system described herein displays a process of selection of components to generate the most stable organized superstructure, representing a control of constitution, structure, and selection driven by self-organization, a factor that may have played an important role in the prebiotic organization of molecular matter.[2a,6] An intriguing consequence would be the generation of a dynamic biopolymer[3d,5d] by selection of those components that lead to the constitution corresponding to the most stable architecture, such as the self-organization-driven selection of amino acid derivatives for a specific proteoid dynamer. Studies are being pursued along these lines.

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[9] See the Supporting Information.
[10] A faint singlet centered at $\delta \approx 8.2$ ppm slowly emerges, which is visible in the $t = 11.8$ h trace of Figure 2a and is thought to arise from a slow irreversible hydrolysis process.
[11] The polymers are insoluble in $CDCl_3$ and CD_2Cl_2. The severe ^{1}H NMR spectral broadening of the polymers in D_2O but not in [D_7]DMF and [D_6]DMSO is taken as further evidence of hydrophobically driven folding.
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