DNA-like "Melting" of Adenine- and Thymine-Functionalized Synthetic Copolymers

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Hydrogen bonding between complementary bases of nucleic acids plays a key role in self-aggregation processes and the spatial arrangement of biological macromolecules such as DNA or RNA.[1] This unique supramolecular performance of nucleic acids has stimulated chemists to explore the possibilities of utilizing these features for the development of advanced artificial systems for various applications.[2,3] In particular, artificial polymers bearing nucleobases moieties are very appealing macromolecules since they can potentially combine the advantages of synthetic functional polymers (low cost, high-yield preparation) and, similarly to DNA, exhibit interesting supramolecular properties.[3] Hence, such synthetic macromolecules are very promising candidates for improvements in molecular nanotechnology[4−7] or biotechnology.[8−10] However, for such new materials, detailed physicochemistry studies of their self-recognition properties are rare.

Herein, we studied a new class of nucleobase functionalized copolymers based on styrene derivatives bearing either thymine or adenine moieties[11] and dodecyl methacrylate. These copolymers were prepared via conventional free-radical copolymerization (the details of the synthesis will be published separately). Dodecyl methacrylate was chosen as a comonomer for two main reasons. First, this monomer possesses long alkyl chains, which allow solubility in a large number of organic solvents. Moreover, the reactivity ratios of methacrylates and styrene derivatives are usually below unity (for example, for styrene and dodecyl methacrylate $r_s = 0.52$ and $r_{DMA} = 0.42$),[12] which suggests a tendency toward alternation of the comonomers during copolymerization. The latter was targeted since it leads to the formation of polymers with a homogeneous chain-to-chain composition (i.e., no composition drift) and a quite regular distribution of nucleobases along each chain. The aim of this work is to show that such artificial copolymers can display a DNA-like melting behavior when complementary adenine (**A**) and thymine (**T**) functionalized polymers are mixed.

The nucleic acid bases **A** and **T** are strong UV chromophores with molar absorptivities between 8×10^4 and 23×10^4 cm^{-1} M^{-1} of their near-UV absorption bands in aqueous solution, and the functional groups involved in H-bonding are part of the chromophoric π systems. Thus, UV/vis spectroscopy is a method of choice for studying the supramolecular association of **coP-A** and **coP-T** (Figure 1). Depending on the atoms that constitute the H-bond donor or acceptor and the respective electronic transition considered, H-bonds can entail various spectral shifts.[13] For nucleic acid base pairs, commonly hypsochromic shifts are found for the n → π^* and bathochromic shifts for the $\pi \rightarrow \pi^*$ transitions.[14,15] Besides spectral shifts due to H-bonding, chromophore−chromophore interactions in an ordered supramolecule can additionally entail hyper- or hypochromic effects as a consequence of interchromophoric coupling.[16] In the case of DNA, both H-bonding and π-stacking are assumed to be important for the formation of the double helix.[17,18]

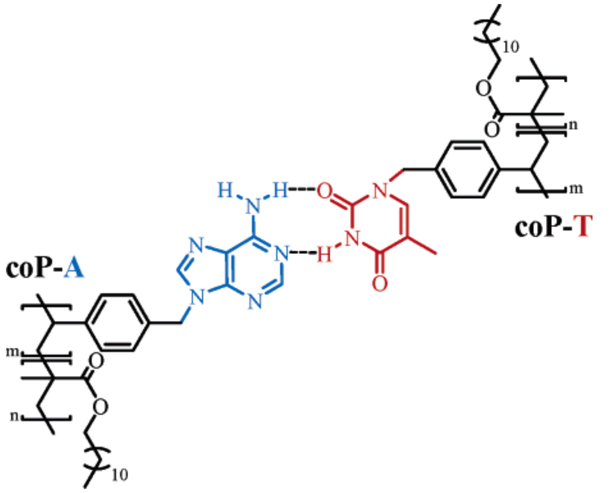

Figure 1. Hydrogen bonds in Watson−Crick paired adenine (**A**) and thymine (**T**) residues as contrived to be present in mixtures of the random copolymers **coP-A** and **coP-T** investigated in this work. **CoP-A** is a copolymer of 9-(4-vinylbenzyl)-adenine and dodecyl methacrylate ($n \sim m$, $M_n \sim 5 \times 10^4$ g mol^{-1}, $M_w/M_n \sim 2.1$). The complementary **coP-T** consists of 1-(4-vinylbenzyl)thymine and dodecyl methacrylate ($n \sim m$, $M_n \sim 3.5 \times 10^4$ g mol^{-1}, $M_w/M_n \sim 2.1$).

Spectrophotometric measurements were carried out in three solvents of different polarity: 1,1,1-trifluoroethanol (TFE), chloroform (CHCl$_3$), and 1,4-dioxane. Both copolymers are sufficiently soluble in the three solvents at concentrations < 1×10^{-4} M (in units of **T** and **A**, respectively), allowing quantitative absorption measurements in conventional 10 mm cells. In the solvent of highest polarity, TFE ($\epsilon = 27.7$), no indication of intermolecular self-aggregation was found. Upon addition of **coP-A** to an equimolar solution of **coP-T**, and vice versa, the resulting absorption spectrum always correlates to a linear combination of the spectra of the single copolymers with the absence of any cooperative effects. The strong ability of protic TFE to act itself as a H-bonding partner prevents association between the complementary copolymers. In CHCl$_3$ ($\epsilon = 4.8$), the picture is more diverse. At low concentrations of the copolymers such as 2.5×10^{-5} M, the absence of H-bonding was also found. At concentrations higher than 4×10^{-5} M, a hypochromic effect typical of nucleic acids self-association was measured,[14,16] suggesting that the onset of intermolecular association of **coP-A** and **coP-T** in this solvent depends on concentration. However, because of the fact that the UV cutoff of CHCl$_3$

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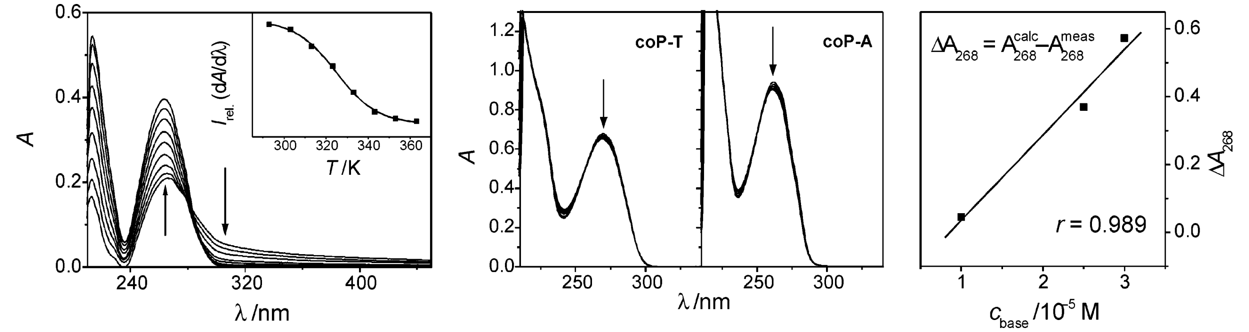

Figure 2. Left: Selected absorption spectra of an equimolar mixture of **coP-T** and **coP-A** in 1,4-dioxane in the temperature range 298−368 K (from bottom to top: 298, 308, 318, 328, 333, 338, 343, 348, and 368 K); $c_T = c_A = 3 \times 10^{-5}$ M. The arrows indicate the changes observed upon increasing the temperature. The position of the longest-wavelength absorption maximum shifts from 267 nm (298 K) via 266.5 nm (308 K) and 265 nm (318 K) to 264 nm (≥328 K). The inset shows the temperature dependence of the intensity at 268 nm of the first derivative absorption spectra of the mixture. (For better visual clearness, different data sets are displayed in the mainframe and the inset.) The latter were analyzed for the determination of the melting temperature to avoid errors due to scattering that contributes to the tailing for the slightly opaque solutions at lower temperatures. Middle: Temperature dependence of the absorption spectra of **coP-A** and **coP-T** in 1,4-dioxane in the same temperature range; $c = 6 \times 10^{-5}$ M. Right: Difference between the calculated (A^{calc})[19] and the actually measured absorbance (A^{meas}) at 268 nm of equimolar mixtures of **coP-T** and **coP-A** in 1,4-dioxane at 298 K as a function of concentration.

lies at rather low energies (245 nm) and strongly overlaps with the lowest energy absorption bands of both **A** and **T** (ca. 260 nm), we refrained from further detailed studies of the copolymers in this solvent. Finally, in the solvent of lowest polarity, 1,4-dioxane ($\epsilon = 2.2$), a distinct hypochromic effect was found for the absorption spectra of a mixture of **coP-A** and **coP-T** over the entire concentration range studied (i.e., $1 \times 10^{-5} \leq c_{base} \leq 3 \times 10^{-5}$ M). As an example, the temperature-dependent absorption spectra of a mixture of 3×10^{-5} M **coP-A** and **coP-T** in 1,4-dioxane are shown in the left panel of Figure 2. The corresponding negligible temperature dependence of the spectra of the individual copolymers is included in the middle panel of the figure. Besides a strongly reduced absorbance in the 260 nm region, the mixture also shows a pronounced tailing on the low-energy side upon base pairing at low temperatures. Additionally, the maxima of the low-energy absorption bands are found at 267 nm for the actual and 264 nm for the calculated mixture of the two copolymers, giving further support to intermolecular **coP-A**−**coP-T** interaction. However, it is important to specify that the present data do not give any information about the structure of the resulting supramolecular aggregates: neither on the number of polymer chains involved in each aggregate nor on the type of base pairs (Watson−Crick model in Figure 1 is only given as an example). Most likely, because of the lack of stereoregularity of the copolymers, the formed aggregates do not possess an ordered secondary structure.

The influence of concentration on the aggregation process is depicted in the right panel of Figure 2. Upon gradual increase of the concentration of both polymers in solution, the difference between the measured and the calculated spectra increases with the trend being correlated. Moreover, even at considerably low concentrations of the bases of 1×10^{-5} M, a hypochromic effect is noticed. These results indicate that even in dilute solutions, the H-bonds between the two copolymers are strong enough to form aggregates in an apolar solvent such as dioxane.

Following the temperature dependence of the absorption spectra of a copolymer mixture in 1,4-dioxane, a pronounced hyperchromic change in the 260 nm region is observed with an isosbestic point at 281 nm (Figure 2, left panel). Additionally, the maximum of the $\pi \rightarrow \pi^*$ transition localized on the nucleobases gradually shifts hypsochromically for 3 nm and is found at 264 nm at 328 K and higher temperatures. At the same time, the spectra of the single copolymers show only minor modifications of an opposite trend (Figure 2, middle panel). The maximum absorbance slightly decreases with increasing temperature with the maximum remaining at 264 nm. Consequently, the negligible influence of temperature on the spectral behavior of the individual copolymers strongly suggests that the single **coP-T** or **coP-A** chains are randomly distributed and oriented in solution and do not form supramolecular associations due to T−T or A−A H-bonding, even in more concentrated solutions (e.g., 6×10^{-5} M) as shown in Figure 2.[20,21] Upon subsequent reduction of the temperature of the solutions containing the mixtures of copolymers from 368 K directly to 298 K, the initial spectrum is not identically reproduced, but the 298 K end point spectrum lies higher. Such effects that are reminiscent of a hysteresis have also been observed for biopolymers.[16] Furthermore, the analysis of the temperature-dependent absorption data of the copolymer mixtures indicates that, similarly to DNA, the aggregates of **coP-A** and **coP-T** also "melt" at a characteristic temperature.[22] The inset of Figure 2, left panel, shows the "melting" curve measured for the most concentrated mixture, and a sigmoidal fit of the data allowed us to determine the "melting" temperature to $T_m = 325$ K (point of inflection). Subjecting the data of the less concentrated mixtures to a similar analysis yields $T_m = 315$ and 320 K for the 1×10^{-5} and the 2.5×10^{-5} M solutions, respectively, reflecting the influence of the overall concentration on the aggregation. Similar observations have been made for other oligomers or homopolymers of nucleobases,[23−26] and these "melting" temperatures are close to that reported for the double-to-single strand transition of poly-dA−poly-dT (325 K).[27]

In conclusion, we have demonstrated that the adenine functionalized copolymer **coP-A** self-assembles with its thymine-functionalized counterpart **coP-T** into supramolecular aggregates, which show a temperature-dependent "melting" behavior in nonpolar solvents. These findings open new possibilities in combining the properties of artificial polymers and biomacromolecules (DNA), for example, for the development of stimuli-responsive drug delivery systems.

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