Cooperativity in the assembly of zipper complexes

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A simple recognition motif comprised of two edge-to-face π–π interactions and one hydrogen bond generates a family of double-stranded zipper complexes from a set of amide oligomers. Cooperativity between the individual recognition sites leads to a substantial increase in the stability of the complexes with increasing oligomer length.

Cooperative interactions between extended arrays of molecular recognition sites are one of the features of biological structures responsible for their stability and selectivity. The simplest systems in which to study such properties are linear oligomeric structures such as double-stranded nucleic acids where there are no direct contacts between recognition sites which are remote in sequence (or covalent structure).[1–6] We recently reported the structure of a molecular zipper **4** composed of two relatively rigid, complementary, linear amide oligomers which form a stable double-stranded complex.[7] Here we show that the basic recognition motif in this system can be used to generate a family of oligomers which form both homodimeric and heterodimeric zippers. Cooperativity in the assembly of these complexes suggests that the binding partners have excellent complementarity and that the zippers could be extended to significantly longer polymeric structures.

The structures of zippers which we have characterised are shown in Fig. 1.† There are two different types of complex: those on the left of Fig. 1 are homodimers of self-complementary molecules; those on the right are 1 : 1 complexes of two different mutually-complementary molecules. All of the complexes have been studied using ^{1}H NMR in $CDCl_3$–CD_3OD (95 : 4 *v*/*v*) solution. Evidence for the stoichiometry comes from Job plots for the heterodimeric complexes, **2** and **4**.[7,8,‡] Evidence for the three-dimensional structures comes from complexation-induced changes in chemical shift in the ^{1}H NMR spectra and NOEs observed in two-dimensional ROESY experiments.[7,8] An X-ray crystal structure of complex **1** has been obtained, and it contains all of the expected features, two edge-to-face π–π interactions and a hydrogen bond.[8]

The complexation-induced changes in ^{1}H NMR chemical shift found by extrapolating titration and dilution curves are similar for all of the complexes, suggesting that they have

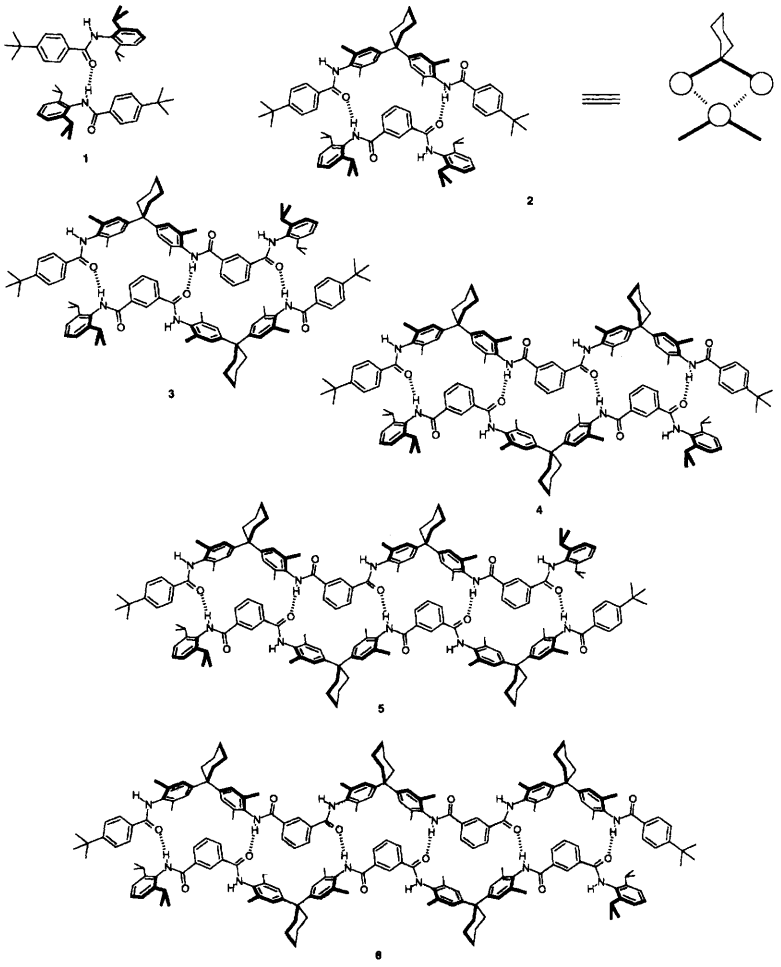

Fig. 1 Structures of the zipper complexes. The complexes on the left are homodimers of self-complementary oligomers. The complexes on the right are 1 : 1 complexes of two different mutually-complementary oligomers (heterodimers). A schematic representation is shown for complex **2**.

similar structures (Fig. 1). The ring current shifts observed for the aromatic protons are indicative of the edge-to-face orientation of the π-systems, and downfield shifts of the signals due to the amide NH protons are characteristic of hydrogen bonds.

Table 1 lists the association constants measured by ^{1}H NMR dilution for **3**, **5** and **6** and ^{1}H NMR titration for **2** and **4**. Complex **1** differs from the other complexes in that it forms extended polymeric assemblies rather than discrete dimeric complexes, because it has two mutually-complementary faces. However, **1** is too weakly bound for an accurate association constant to be determined. Although the longer zippers are more stable than the shorter zippers, there is no uniform trend in the values in Table 1: *e.g.* complex **3** has almost the same

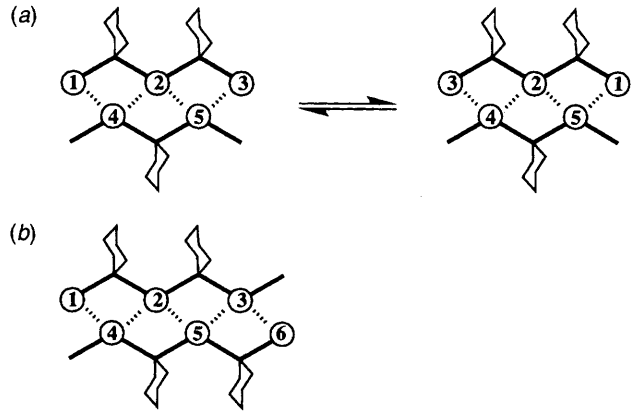

Fig. 2 Schematic representations of (*a*) the heterodimeric zipper **4** and (*b*) the homodimeric zipper **5**. The isophthaloyl groups in each complex are numbered to allow differentiation of degenerate conformations. The heterodimeric complexes can adopt two different types of conformation whereas the homodimeric zippers can only adopt one.

Table 1 Association constants (K_a) and corresponding free energies of binding (ΔG_b) determined from ^{1}H NMR titration and dilution experiments in $CDCl_3/CD_3OD$ (95/5 *v/v*) at 298 K

Complex	K_a/dm^3 mol^{-1}	ΔG_b/kJ mol^{-1}
2	$1.8 \pm 0.3 \times 10^1$	-7.2 ± 0.4
3	$2.0 \pm 0.3 \times 10^1$	-7.5 ± 0.4
4	$2.4 \pm 0.1 \times 10^2$	-13.7 ± 0.1
5	$1.1 \pm 0.2 \times 10^3$	-17.4 ± 0.4
6	$5.5 \pm 3.5 \times 10^4$	-27.2 ± 1.6

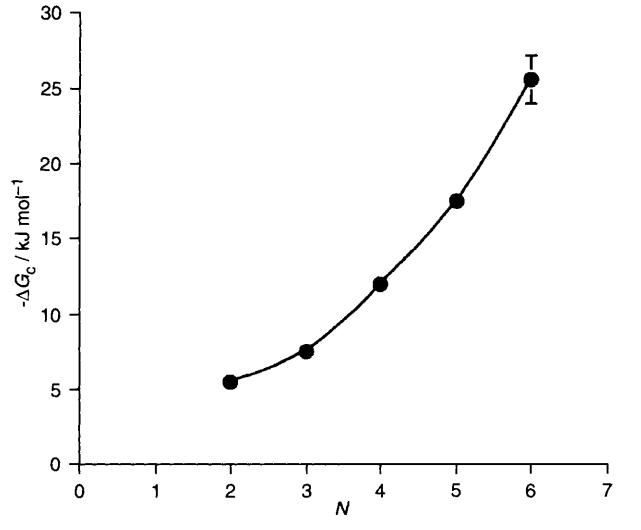

Fig. 3 The statistically-corrected free energy of binding in $CDCl_3/CD_3OD$ (95/5 *v/v*) ($-\Delta G_c$) plotted against the length of the oligomer (*N*). For homodimeric zippers $-\Delta G_c = -\Delta G_b$, and for heterodimeric zippers $-\Delta G_c = -\Delta G_b/2$. N is the number of repeats of the recognition motif (two edge-to-face π–π interactions and one hydrogen bond). Error bars for most of the points lie within the symbol.

association constant as complex **2**. In order to make sense of this data, the symmetry of the complexes must be considered. Fig. 2 shows that the heterodimeric complexes can adopt twice as many degenerate conformations as the homodimeric complexes formed by the self-complementary oligomers. Thus a statistical factor of two must be taken into consideration when comparing the stabilities of the two types of complex. Fig. 3 shows a plot of the statistically-corrected free energy of binding *vs.* length of the oligomer: now, a smooth trend is obtained.

The relationship between complex stability and length is not linear but curved upwards. In other words, the cooperativity between the individual recognition sites increases in the longer zippers compared with the shorter less stable complexes. There are several possible explanations. (1) There is a mismatch between the optimum orientation of the recognition sites and the geometry of the covalent connections in the oligomers. Thus the binding interactions are optimised in complex **2**, but there is a mismatch in complex **3** which is gradually dissipated in the longer oligomers. (2) The zippers contain no sequence information, and so a number of alternative structures may be populated for each structure. Both slipped and frayed structures which do not contain the full complement of hydrogen bonds are possible. As the length of the oligomer increases, the number of possible alternative structures increases. Simulations based on this model do lead to an upward curve in the $-\Delta G_c$ *vs.* *N* plot, but the curvature is less pronounced than observed experimentally. (3) When an intermolecular complex forms there is some residual entropy present due to intermolecular vibrational modes. As the overall stability of the complex increases, this residual entropy decreases until a limit is reached in the loss of translational and rotational entropy of one of the binding partners involved in complexation.[9,10] (4) Williams has suggested that the enthalpy of individual binding interactions increases in strongly bound complexes compared with weakly bound complexes.[11]

The complexation-induced changes in ^{1}H NMR chemical shift found in the zipper complexes do not show any $-\Delta G_c$ dependence which suggests that (3) is the most likely explanation for the curvature. However, more detailed thermodynamic measurements are required before we can rule out the other effects.

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Footnotes

† All new compounds gave satisfactory spectroscopic data.

‡ In principle, these systems may form larger oligomeric aggregates. Our experiments show that this does not happen for the shorter zippers but do not exclude this possibility for the longer zippers which are more difficult to characterise.

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