Refoldable Foldamers: Global Conformational Switching by Deletion or Insertion of a Single Hydrogen Bond
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Abstract: Small changes in the structure of a foldamer may lead to gross changes in conformational preference. We show that the simple insertion or deletion of a single hydrogen bond by changes in pH or by photochemical deprotection is sufficient to refold a helical oligomer, interconverting M and P screw-sense preference. As a consequence of the switch, information may be transmitted to a remote catalytic site, selectively directing the formation of either of two enantiomeric products by a reaction involving 1,2,2-remote intermolecular asymmetric induction.

The higher order structure of proteins and peptides is generally remarkably tolerant of modifications of primary structure: the serine proteases, for example, display similar tertiary structures and have near-identical functions across a wide range of organisms, despite variations in their primary sequence of up to 50%.

Here we show that even a change as minimal as insertion or deletion of a single hydrogen bond, initiated by a photochemical switch or a change in pH, may lead to a global conformational switch in a group of peptide-like foldamers. The foldamers in question were designed to favour strongly a global helical conformation by building them from the powerfully helicogenic quaternary amino acid Aib. Homooligomers of Aib of greater than three residues are essentially entirely 3_{10} helical, especially in non-polar solvents.

Oligomers of Aib diverge subtly from Gellman’s original definition of a foldamer, since they populate not one but two principal conformations of opposite screw sense: in an entirely achiral oligomer these rapidly interconverting conformers are enantiomeric and therefore necessarily isoenergetic.

The two screw-sense conformers of an Aib oligomer may be desymmetrised by ligation to a chiral terminus—which leads to an imbalance in the population of the two interconverting screw-sense conformers. This imbalance may be quantified by NMR, and a C terminal AlaNHR residue, for example, may induce a 99:1 preference for right-handed screw sense in an attached Aib oligomer chain.

The ability of a terminal residue to induce a screw-sense preference is allied to the way in which it organises the final β turn of the 3_{10} helix, and we set out to devise systems in which the simple addition or deletion of a hydrogen bond was sufficient to lead to a fundamental change in the structure of this structure and hence a global modification of conformation.

Oligomer 4 bearing Ala as a C terminal inducer of screw-sense preference was synthesised as shown in Figure 1. The C terminal hydrogen bond of the Ala residue was “erased” chemically by functionalisation as a tertiary amide derivative using the photosensitive 5-bromo-7-nitroindoline (Bni) 1, which was ligated to L-alanine without racemisation to give 2 as a coupling partner. Alaninamide 2 was coupled to an Aib pentamer 3 in which the N terminal residue was enantiosselectively isotonically enriched with ^{13}C in its pro-R and pro-5 methyl groups in a 75:25 ratio, giving the helical oligomer 4.

VT ^{13}C NMR studies of 4 showed that it displayed a 72:28 preference for a left-handed screw sense at 25°C in THF (Figure 1a). The left-handed helical oligomer 4 was irradiated at 360 nm for 1 h in [D_2]THF in the presence of an excess of isopropylamine. Under these conditions, the tertiary amide fragments to a reactive O-acyl nitrate, which is trapped by the amine to form secondary amide fragments to reactive

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at the C-terminus of the oligomer,\textsuperscript{[25]} inducing the formation of new β-turn (as shown for 5) in place of the extended structure at the C-terminal of 4, and thus the global reversal of screw-sense preference.

The selective deletion or re-formation of a terminal hydrogen bond could alternatively be achieved by removal or addition of a single proton by treatment with base or acid. Achieving the regioselectivity necessary for selective deprotonation/reprotonation of a compound containing numerous N–H bonds requires the incorporation of a structure that induces a substantial decrease in \( pK_a \) of a specific N–H group. Metal ions such as Ni\textsuperscript{2+} or Cu\textsuperscript{2+} coordinate amino acid residues in peptides through their amide N atoms, acidifying the amide group and lead to spontaneous deprotonation.\textsuperscript{[42,43]} A metal binding site was thus ligated to the C-terminus of foldamer 8 with the aim of localising binding of a metal to the C-terminal amide linkage and promoting deprotonation of the C-terminal N–H (Figure 2, shown in orange). To give well-defined monomeric structures, the tridentate tren structure 6 was chosen as a binding site to satisfy three of the metal ion’s coordination sites. Tetramine 6 was selectively doubly Boc protected at its primary amino groups\textsuperscript{[44]} and ligated to L-alanine to yield 7, which was coupled to labelled foldamer 3 to give 8.

In order to allow analysis by NMR, the C-terminal amide NH bond of 8 was acidified with Zn\textsuperscript{2+} (rather than Cu or Ni) cations.\textsuperscript{[41]} Zn(ClO\textsubscript{4})\textsubscript{2}·6H\textsubscript{2}O was added in portions to a solution of the oligomer 8 in CD\textsubscript{3}OH. \( ^1\text{H} \) NMR (Figure 2a) showed a significant change in chemical shift of only one NH, identified as the C-terminal NH of the helix by its triplet multiplicity. The signal migrated from 7.78 to 9.02 ppm on addition of 1 equiv of Zn(ClO\textsubscript{4})\textsubscript{2}, but shifted very little on addition of 2 equiv of Zn(ClO\textsubscript{4})\textsubscript{2}.
further addition of the salt, confirming the 1:1 metal binding stoichiometry. The $^{13}$C spectrum of the complex (Figure 2b) showed that the oligomer retained its right-handed screw sense.

The downfield shift suggests acidification of the NH group by coordination of the terminal amide to Zn, but not spontaneous deprotonation. The complex formed from 8 and 1.1 equiv Zn(ClO$_4$)$_2$·6H$_2$O was therefore treated with Bu$_3$NOH (1.1 equiv), a base with a non-coordinating counterion. As a result, the NJCH$_2$ group disappeared from the $^1$H NMR spectrum (Figure 2a). The $^{13}$C NMR spectrum also showed a change in the position of the major and minor $^{13}$C labels, indicating a switch from in screw sense from $P$ in 8H·Zn$^{2+}$ (Figure 2b, with anisochronicity 1966 ppb, corresponding to 9:91 screw-sense preference) to $M$ in 8Zn$^+$ (Figure 2c, anisochronicity 860 ppb, corresponding to 68:32 screw-sense preference). Neutralisation of the base with acetic acid (1.1 equiv) gave a $^{13}$C NMR spectrum (Figure 2d) similar to that of 8H·Zn$^{2+}$, indicating complete restoration of the original screw-sense preference. A second addition of base (Figure 2e) and then acid (Figure 2f) confirmed the repeatability of the pH-directed refolding process, allowing interconversion of the two conformers.

Natural conformationally switchable proteins such as rhodopsin and other G-protein coupled receptors function by setting in progress a chemical transformation as a result of a conformational change.$^{[45,46]}$ In order to allow synthetic analogues related to 4 and 8 the potential to translate a conformational change into a detectable change in chemical reactivity, we devised a range of achiral catalytic sites based on modifications of Takemoto’s aminothiourea catalysts$^{[47]}$ and appended them to the N terminus of a right-handed helical structure bearing a C terminal alanine residue. The resulting foldamers 9a-f contain a catalytically active site with no local chirality, whose conformation may nonetheless be induced to be asymmetric by a remote stereogenic centre two helical turns, or 13–16 bonds, away. These foldamers were used to catalyse the addition of dimethyl or diethyl malonate to β-nitrostyrene (Figure 3 and Table 1). Only the less sterically hindered catalysts 9e and 9f were successful (entries 5–7); conversion with 9a–9d was low (entries 1–4), irrespective of an anticipated beneficial Thorpe–Ingold effect.$^{[48]}$ Despite the spatial separation of the catalytic site from the source of asymmetry, enantiomeric ratios of ca. 75:25 were obtained in favour of the $R$ enantiomer of the product 10, rising to 82:18 with dimethylmalonate as the nucleophile (entry 7). A control experiment (entry 8) in which the catalytic site and the asymmetric centre were located in different molecules demonstrated that the asymmetric induction was the result of intramolecular conformational induction: a 1:1 mixture of thiourea 9g and CbzGlyAlb$_2$AlaNH$_2$Bu 9h catalysed the formation of 10 in 68% yield in essentially racemic form.

We propose that the asymmetric addition to nitrostyrene proceeds through a transition state approximating to that illustrated in Figure 4.$^{[49]}$ Hydrogen bonding between the acidic malonate and the piperedine function at the N terminus of the $P$ helix allows attack on the lower face of the thiourea-bound nitrostyrene with the malonate ester substituents and nitrostyrene phenyl group avoiding the steric clash that would arise from attack on the other face of the nitrostyrene were inverted. The preferential formation of ($R$)-10 involves organocatalysis proceeding with unprecedentedly remote$^{[33,36,51]}$ intermolecular 1,22 asymmetric induction.

The optimal catalytic site of 9f was coupled to an oligomer carrying at its C terminus the photoswitchable AlaBni residue, giving foldamer catalyst 11. Addition of this catalyst to a mixture of dimethylmalonate and nitrostyrene in dichloromethane gave, after 18 h, ($S$)-10 in 37:63 er (Figure 5). The catalyst was then irradiated at 360 nm in the
presence of isopropylamine, converting the tertiary amide into a secondary amide and inducing the helix to refold into a left-handed conformation. The catalyst was again added to a mixture of dimethylmalonate and nitrostyrene in dichloromethane to give, after 72 h, a 78% yield of \((R)-10\) in 77:23 ee.

Asymmetric organocatalysts with stereoselectivity switchable \(^{[32]}\) by light, \(^{[33]}\) by solvent \(^{[34]}\) or electrochemically \(^{[35]}\) have been reported, as have catalysts based on helical polymers with invertible screw sense. \(^{[36,37]}\) In this “allosteric” example, the switchable source of asymmetric induction is remote from the site of catalysis and its stereochemical influence is relayed unidirectionally through a helical chain. The induced remote reversal of enantioselectivity demonstrates that insertion of a single hydrogen bond at a remote site in a refoldable molecule is sufficient to induce a global conformational switch that translates information about a small change in structure into a chemically significant, detectable result.

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[38] Coupling of 2 to Cbz-l-Phe gave a single diastereoisomer by 'H and 13C NMR.
[40] Conformational ratios were estimated by our reported method whereby a 13C NMR chemical shift difference Δδfast at room temperature (when the rate of helix inversion is fast on the NMR timescale) is divided by a 13C NMR chemical shift difference Δδslow at a temperature below the coalescence temperature. The resulting value corresponds to “helical excess” from which the ratio of screw-sense conformers is calculated. See Refs.[24,27].
[41] The screw sense of the major conformer was deduced from the relative positions of the 13C NMR signals corresponding to the enantioselectively isotopically labelled diastereotopic methyl groups of the R-Aib* residue. For an N-terminal CbzAib* label, the major signal is upfield in a P helix and downfield in a M helix. See Refs.[25,26,33].

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