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Cyclic Hexapeptide of D,L-α-Aminoxy Acids as a Selective Receptor for Chloride Ion

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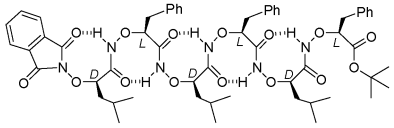
In recent years, significant effort has been devoted to the investigation of the functions of foldamers, that is, oligomers of unnatural building blocks that fold into defined secondary structures.[1] For example, oligomers of β-amino acids (β-peptides) with significant antimicrobial activities have been reported.[2] Cyclic peptides composed of D,L-α-amino acids or β^3-amino acids have been discovered to self-assemble into nanotubes and function as transmembrane ion channels.[3] These works inspired us to investigate α-aminoxy acids, a class of unnatural amino acids, for their potential functions. Our previous work demonstrated that, as a backbone analogue of β-amino acids, α-aminoxy acids represented a new class of foldamers. They can induce an eight-membered-ring intramolecular hydrogen bond (the N−O turn)[4] between adjacent residues when incorporated into peptides, and oligomers of D-α-aminoxy acids can form right-handed 1.8_8 helical conformations in nonpolar solvents.[5] Here we report that cyclic hexapeptide of D,L-α-aminoxy acids can function as a selective receptor for chloride ion.



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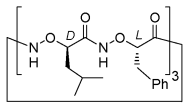

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Linear hexapeptide **1** of alternating D- and L-α-aminoxy acids was prepared following our previously developed convergent synthetic scheme.[5a] The ^{1}NMR study in $CDCl_3$ showed that the chemical shifts of all amide NHs of **1** were rather downfield (10.6−11.5 ppm) and independent of concentrations,[6] indicating that they were intramolecularly hydrogen bonded with consecutive N−O turns. Hexapeptide **1** was then deprotected at both ends and treated with PyAOP[7] to give cyclic hexapeptide **2** in 35% yield. In the ^{1}H NMR spectra of **2** in $CDCl_3$,[6] only two sets of sharp peaks originating from the two aminoxy acid residues were observed. The downfield signals of amide NHs of **2** (at 11.90 and 11.84 ppm) were almost unchanged in the concentration range of 0.5−200 mM, characteristic of intramolecular hydrogen bonds. This was also corroborated by the fact that the N−H stretch frequency of **2** appeared at a lower wavenumber (3182 cm^{-1} at 1 mM in CH_2Cl_2) than that of a typical non-hydrogen-bonded aminoxy amide N−H (3400−3380 cm^{-1}). Furthermore, it was found that in non-hydrogen-bonding solvents, the NOE pattern[6] of **2** was in accordance with those of N−O turns: strong NOE between NH_i and $C_\alpha H_i$ but weaker NOE between NH_{i+1} and $C_\alpha H_i$.[4b,5a] Therefore, **2** should adopt cyclic conformations with consecutive N−O turns.

A conformational search of model cyclic hexapeptide **3** (with all methyl side chains) resulted in **3a** as the global minimum (Figure 1).[6] The backbone of **3a** folds into consecutive N−O turns with strong hydrogen bonds (d(H···O) = 2.05 Å, ∠N−H···O = 140°) and adopts a C_3 symmetric and bracelet-like conformation, in agreement with the fact that **2** only gave two sets of amide NH peaks in its ^{1}H NMR spectra. All α-protons of aminoxy acid residues point inward, and the side chains point outward with those of D-aminoxy acids on one face of the bracelet and those of L-aminoxy acids on the other face. It is therefore conjectured that **2** has a polar interior and a nonpolar exterior. This is supported by the fact that **2** has excellent solubility in nonpolar organic solvents such as dichloromethane, diethyl ether, and benzene. The preferred conformation of **3** is reminiscent of that of cyclodepsipeptide valinomycin, a natural ionophore with a high selectivity for K^+ ion.[8] By forming consecutive 10-membered-ring intramolecular hydrogen bonds, valinomycin also has a bracelet-like conformation in nonpolar solvents.[8]

Given its bracelet-like conformation and small pore size (d = 3.22 Å from calculated structure of **3a**), **2** is expected to bind small ions. Its carbonyl groups may coordinate with cations, whereas the amide NHs may form intermolecular hydrogen bonds with anions. Thus, both cations and anions were screened for possible complexation with **2** by using ^{1}H NMR titration and ESI-MS methods. Interestingly, **2** was found to bind halide ions[9−11] but not alkali metal ions.[12]

Figure 2 shows the amide proton region of ^{1}H NMR spectra of free **2** and the 1:1 mixture of **2** with Cl^-, F^-, and Br^-, respectively, at −30 °C in $CDCl_3$. The two singlets at about 12 ppm corresponded to the amide NHs of free **2**, and the two singlets at about 11 ppm were assigned to amide NHs of halide-bound **2**. Clearly, the selectivity of **2** for halide ions follows the order of $Cl^- \gg F^- \gg Br^-$.

In the ESI-MS spectra (−ve mode) of a 1:1 or a 1:2 mixture of **2** and Ph_4PCl, only two peaks (m/z 876.5 and m/z 911.9) were observed, corresponding to free **2** and the 1:1 complex of **2** and Cl^-, respectively. The abundance of **2**·Cl^- complex was also much higher than that of free **2**.[6]

The association constants K_a for complexes **2**·Cl^- and **2**·F^- were then calculated to be 11880 and 30 M^{-1}, respectively, at 298 K using the van't Hoff plots of K_a values obtained from low temperature ^{1}H NMR experiments.[6] Considering the pore size of **3a** and the diameters of halides, we found that Cl^- (d = 3.34 Å) is more suitable than F^- (d = 2.38 Å) and Br^- (d = 3.64 Å) for binding **2**.[13] However, the hydrogen-bonding ability of halides follows the sequence of $F^- > Cl^- > Br^-$. Our experimental results suggested that the selectivity of **2** for halides is mainly governed by the size complementarity rather than the hydrogen-bonding strength.

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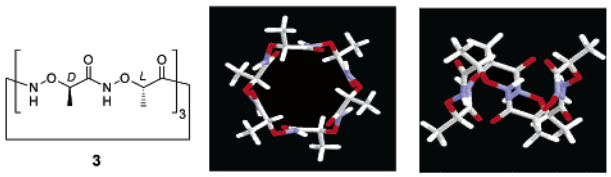

Figure 1. HF/6-31G* optimized lowest-energy conformation of model cyclic hexapeptide **3**.

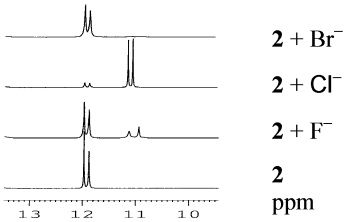

Figure 2. The amide NH region of overlaid ^{1}H NMR spectra of free **2** and the 1:1 mixture of **2** with $(n\text{-Bu})_4$NF, Ph_4PCl, and Ph_4PBr, respectively (3 mM in $CDCl_3$ at −30 °C).

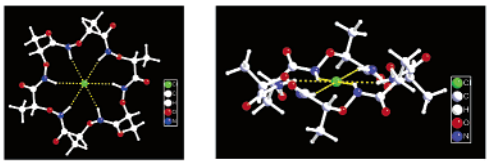

Figure 3. The HF/6-31G* optimized lowest-energy conformation of **3**·Cl^-: top view (left) and side view (right).

The solution conformation of **2**·Cl^- complex was then investigated using ^{1}H NMR techniques. In CD_2Cl_2, only one set of sharp peaks was found in the ^{1}H NMR spectrum of a 1:2 mixture of **2** (6 mM) and Ph_4PCl at room temperature.[6] The ^{1}H NMR spectrum also indicated that **2**·Cl^- complex still adopted a C_3 symmetrical conformation. The HMBC spectrum revealed that amide NHs of OLeu and OPhe switched their positions in the ^{1}H NMR spectrum after **2** was complexed with Cl^-. The ROESY spectrum exhibited a different NOE pattern (medium NOEs between NH_i and $C_\alpha H_i$ and also medium NOEs between $C_\alpha H_i$ and NH_{i+1}) as compared with that of free **2** under the same condition, indicating that the conformation of **2**·Cl^- was quite different from the bracelet-like conformation.

The HF/6-31G* optimized[6] lowest-energy conformation of **3**·Cl^- complex is shown in Figure 3. Upon Cl^- ion binding, the original bracelet-like conformation **3a** turns into a rather flat conformation with all of the amide NHs pointing inward. The Cl^- ion at the center of **3** is hydrogen bonded to six NHs simultaneously. The calculated H···Cl distance and N−H···Cl angle are 2.40 Å and 154°, respectively. The distance between NH_i and $C_\alpha H_i$ is 3.19 Å, whereas that between $C_\alpha H_i$ and NH_{i+1} is 3.40 Å, matching well with the observed NOEs for **2**·Cl^- complex in solution.

In conclusion, we found that cyclic hexapeptide **2** consisting of alternating D,L-α-aminoxy acids adopted the highly symmetrical bracelet-like conformation, which is quite different from those of cyclic D,L-α-peptides and β^3-peptides but similar to that of valinomycin. In contrast to valinomycin that binds cations selectively, **2** showed affinities for halides with high selectivity for Cl^- ion in nonpolar solvents. The discovery of a novel chloride receptor may open up new opportunities in the molecular design of selective chloride sensors and transporters.

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Supporting Information Available: Synthetic scheme and characterization data of **1** and **2**; ^{1}H NMR studies of **1** and **2**; ^{1}H NMR and ESI-MS spectra of mixtures of **2** and Ph_4PCl; determination of the association constants for **2**·Cl^- and **2**·F^- complexes; calculation procedures and the calculated energies and coordinates of **3a** and **3**·Cl^- complex (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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