Size-dependent cation transport by cyclic α-peptoid ion carriers†

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Received 20th March 2009, Accepted 22nd May 2009
First published as an Advance Article on the web 29th May 2009
DOI: 10.1039/b905665c

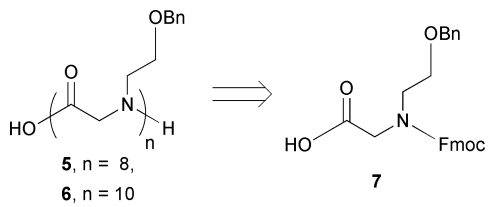
***N*-Benzyloxyethyl macrocyclic peptoids 3 and 4 were synthesized and subjected to alkali metal binding studies; these compounds, plus the known 1 and 2, when subjected to ion transport studies, demonstrated size-dependent selectivity for the first group alkali metals cation transport.**

Transport of metal ions in biological systems is a function essential to life. It relies on immensely complex polypeptides, whose architecture specifies ion capture and conduction.[1] Cation recognition and transport can also be achieved through small size host molecules: in some cases their ion affinities and permeation abilities are comparable to the natural multimeric pores.[2] A survey of the structurally diverse ionophores demonstrates that the cyclic arrangement represents a common archetype equally promoted by chemical design[3] and evolutionary pressure.[4] Cyclization of linear precursors is, in fact, a successful strategy to obtain coordination geometries similar to those found in the highly conserved selectivity filters.[1]

In this context, cyclic peptoids[5] belonging to the vast realm of bioinspired pseudopeptides, appear as an ideal platform for the design of new ionophores. In a previous investigation[5a] we found, for the flexible eighteen-membered *N*-benzyloxyethyl cyclic peptoid **2**, high binding constants with the first group alkali metals ($K_a \sim 10^6$ for Na^+, Li^+ and K^+), while, for the rigid *cis–trans–cis–trans* cyclic tetrapeptoid **1**, there was no evidence of alkali metals complexation.

On the basis of these partial results, we decided to explore the full potentials of the α-cyclopeptoids in the field of cation complexation and ionophoric activity toward first group alkali metal ions. Herein we report our investigations on the synthesis and the complexation properties displayed by the cyclooctapeptoid **3**, and cyclodecapeptoid **4**. We also describe the first results on the group I cations transport studies for the four cyclic peptoids **1–4**.

The synthesis of the linear *N*-benzyloxyethyl glycine oligomers was accomplished on solid-phase (2-chlorotrityl resin) using the "monomer" approach. Linear octamer (**5**) and decamer (**6**) peptoids were prepared with considerable purity (>95%, RP-HPLC and ESI-MS analysis, see ESI†), by stepwise incorporation of the known *N*-fluorenylmethoxy-carbonyl,*N'*-benzyloxyethyl glycine (**7**)[5a] in the presence of the phosphonium- or guanidinium-type coupling reagents PyBOP or HATU (for both the condensing agents the average coupling yield was >96%).



The oligomers **5** and **6** were then efficiently cyclized (high dilution conditions: $2.0 \cdot 10^{-3}$ M) with the assistance of PyBOP, giving the expected targets **3** and **4** in good yields (>90%, RP-HPLC analysis, see ESI†).

The complexity of the r.t. ^{1}H NMR spectrum recorded for the cyclic **3**, demonstrated the slow exchange of multiple conformations on the NMR time scale (Fig. 1a).

Stepwise addition of sodium picrate to **3**, induced the formation of a complex with a remarkably simplified ^{1}H NMR spectrum (Fig. 1b–c). With an excess of guest, we observed the formation of an 8-fold symmetric species (Fig. 1c).

An extensive conformational search on **3**, performed without sodium cations, followed by a quantum chemical geometry optimization at DFT/HF ONIOM level[6] (see ESI†), suggested the presence of a rare S_8-symmetry axis (Fig. 2).[7]

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† Electronic supplementary information (ESI) available: Experimental section (synthesis, complexation studies, computational details, ion transport data) with the list of abbreviations. See DOI: 10.1039/b905665c

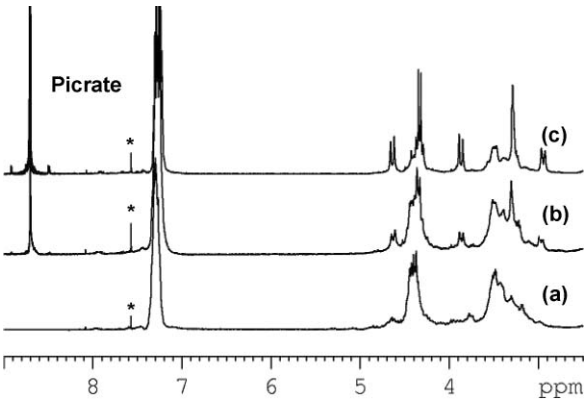

Fig. 1 ^{1}H NMR spectra of free **3** (a) ($CD_3CN/CDCl_3$ 9:1 solution, 25 °C, [**3**] = 4.0 mM, 400 MHz) and in the presence of 2.0 eq. (b) or 10.0 eq. (c) of sodium picrate. The residual solvent peak ($CHCl_3$) is labelled with *.

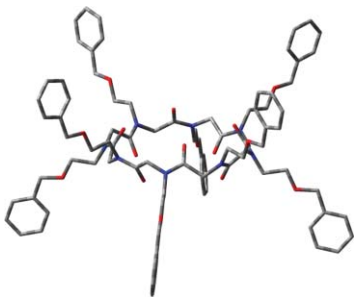

Fig. 2 Picture of the predicted lowest energy conformations for **3** without sodium cations. The peptoid bonds display an all-*trans* geometry (atom type: C gray, H white, N blue, O red, Na magenta). Hydrogen atoms omitted for clarity.

Differently from the twenty-four-membered **3**, the *N*-benzyloxyethyl cyclic homologue **4** did not yield any ordered conformation in the presence of cationic guests.[8]

The association constants (K_a) for the complexation of **3** and **4** to the first group alkali metals and ammonium, were evaluated in $H_2O/CHCl_3$ following the Cram's method (Table 1).[9] The results presented in Table 1 show a good degree of selectivity for the larger cations, with a peak for Cs^+ in the case of cyclic α-peptoid **3**, and a slight preference for K^+ in the case of **4**.[10]

Table 1 R, K_a, and ΔG° for cyclic peptoid hosts **3** and **4** complexing picrate salt guests in $CHCl_3$ at 25 °C; figures within ±10% in multiple experiments, guest/host stoichiometry for extractions was assumed as 1:1

Host **3**:			
Picrate salt	R[a]	K_a [M^{-1}]	−ΔG° [kcal/mol]
Li^+	0.14	0.66×10^6	7.9
Na^+	0.17	0.73×10^6	8.0
K^+	0.26	1.1×10^6	8.2
Rb^+	0.25	1.3×10^6	8.3
Cs^+	0.29	15×10^6	8.4
NH_4^+	0.25	0.65×10^6	7.9
Host **4**:			
Picrate salt	R[a]	K_a [M^{-1}]	−ΔG° [kcal/mol]
Li^+	0.13	0.58×10^6	7.9
Na^+	0.14	0.55×10^6	7.8
K^+	0.23	1.2×10^6	8.3
Rb^+	0.19	0.73×10^6	8.0
Cs^+	0.21	0.77×10^6	8.0
NH_4^+	0.18	0.39×10^6	7.6

[a] [Guest]/[host] in $CHCl_3$ layer at equilibrium.

The ability of cyclic peptoids to extract cations from bulk water to an organic phase prompted us to verify their transport properties across a phospholipid membrane. The two processes are clearly correlated although the latter is more complex implying, after complexation and diffusion across the membrane, a decomplexation step.[11] The ionophoric properties of cyclic peptoid **1**–**4** were investigated using the pH-sensitive fluorescent dye HPTS (HPTS = 8-hydroxypyrene-1,3,6-trisulfonic acid, pK_a = 7.2).[12] In this assay the dye is entrapped inside phospholipid vesicles buffered at pH = 7 and, after addition of the ionophore, a pH gradient of 0.6 pH units is applied by external addition of NaOH. The collapse of this transmembrane pH-gradient implies basification of the liposome inner water pool which is signalled by an increase of the HPTS fluorescence emission. This basification process may derive either from H^+ efflux or OH^- influx and this transmembrane charge translocation needs to be counterbalanced leading to four possible overall processes: H^+/Na^+ antiport, OH^-/Cl^- antiport, H^+/Cl^- symport and Na^+/OH^- symport. The ionophoric activity of the cyclic peptoids is reported in Fig. 3a.

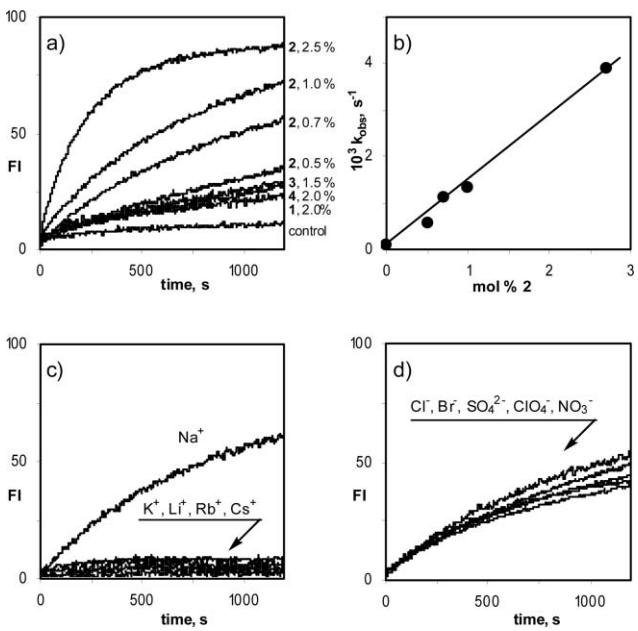

Fig. 3 (a) Normalized fluorescence change in HPTS emission (FI, λ_{ex} 460 nm, λ_{em} 510 nm) as a function of time after addition of the base (50 μL of 0.5 M NaOH) to 95:5 EYPC/EYPG LUVs (100 nm diameter) loaded with HPTS (0.1 mM HPTS, 0.17 mM total lipid concentration, 25 mM HEPES, 100 mM NaCl, pH 7.0, total volume 3 mL), in the presence of cyclic peptoids **1**–**4**. The concentration of the ionophore is indicated in the Figure and is given in percent with respect to the total concentration of lipids. (b) Dependence of k_{obs} on the concentration of cyclopeptoid **2**. (c) Cation selectivity for cyclopeptoid **2** (1% concentration), using the HPTS assay (100 mM MCl, pH 7.0, base pulse by addition of 50 μL of 0.5 M MOH). (d) Anion selectivity for cyclopeptoid **2** (0.7% concentration), using the HPTS assay (100 mM NaX, pH 7.0, base pulse by addition of 50 μL of 0.5 M NaOH). The kinetic traces of panels 3c and 3d are corrected for the permeation of the anion or cation under investigation in the absence of ionophore. (HEPES: 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid; EYPC: egg yolk phosphatidyl choline; EYPG: egg yolk phosphatidyl glycerol).

In the presence of NaCl as added salt (Fig. 3a), only compound **2** shows ionophoric activity while the other cyclopeptoids are almost inactive. Fitting of the kinetic traces obtained for different concentrations of **2** gives the apparent first-order rate constants (k_{obs}, s^{-1}) for the transport process, which are reported in Fig. 3b. Increasing the concentration of ionophore, the rate of transport increases linearly and this behavior is in accord with a carrier-based transport mechanism. Interestingly, the efficiency of transport strongly depends on the cation and not on the anion present. As shown in Fig. 3c, which reports experiments with ionophore **2** in the presence of chloride salts of the alkaline cations, only with Na^+ ion the transport is active. On the contrary, the process is almost unaffected by the anion present (as Na^+ salt) and the same rate of transport is observed with anions of different type, size, charge and lipophilicity. Clearly, anions are not involved in the process and the activities recorded in Fig. 3d are related to the transport of Na^+ which is present as a countercation of all the investigated anions. Therefore, the pH-gradient discharge process is apparently governed by the transport of Na^+ promoted by the cyclopeptoid. Support to this hypothesis comes from ^{23}Na-NMR experiments which show that compound **2** is able to transport efficiently Na^+ across a phospholipid membrane even in the absence of a pH-gradient (see ESI†).

Cyclic peptoids have different cation binding preferences and, consequently, they may exert selective cation transport. Fig. 4 reports the cation selectivities in the transport process as determined from the HPTS assay.

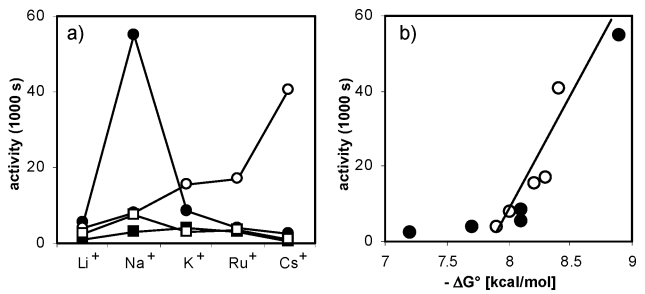

Fig. 4 (a) Cation selectivities determined for the cyclic peptoids **1** (■, 2%), **2** (●, 1%), **3** (○, 0.75%), and **4** (□, 2%) in the conditions of Fig. 3c. Due to the difficulty of obtaining a reasonable fitting for the slower kinetics, the Figure reports the activity measured at 1000 s corrected for the unassisted permeation of the cation and for the different concentration of ionophore used (simply by dividing the observed activity for the ionophore concentration). The original kinetic profiles are reported in the ESI.† (b) Dependence of the activity of compounds **2** (●) and **3** (○) in the transport of the alkaline cations on the ΔG° for the complexation of the corresponding picrate salt in $CHCl_3$ at 25 °C. The ΔG° values for compound **2** are taken from ref. 5a.

While **1** and **4** are substantially inactive with any cation, cyclic peptoids **2** and **3** show a size-dependent selectivity, with transport preferences for Na^+ and Cs^+, respectively. For these two compounds the transport activity correlates with their cation extraction ability suggesting that the complexation step is also relevant in the transport process (Fig. 4b). Furthermore, the data of Fig. 4b indicate that a detectable transport rate is observed only when the $-\Delta G°$ of extraction is above about 8.2 kcal/mol. In the case of lower cation extraction ability, the transport, if present, is too slow and the unassisted proton permeation dominates the overall process. This explains the inactivity of **1**, which is essentially unable to extract any cation[5a] and, at least partially, also that of **4**. However, for this compound a role may also be played by the different conformations[8] of the complexes that may somehow affect the interactions with the phospholipid membrane.

The efficacy of **2** as ionophore is strongly dependent on the membrane fluidity. The activity observed in EYPC/EYPG vesicles at 25 °C, where the membrane is in the fluid-phase, is completely suppressed in DPPC (DPPC = 1,2-dipalmitoyl-s,n-glycero-3-phosphocholine) vesicles which, at the same temperature, are in gel-phase (see ESI†). Such strong dependence of the transport rate on the fluidity of the bilayer is classical evidence of a carrier-based mechanism[13] which complements the above observations regarding the linear dependence of the rate/concentration profile and the correlation between the transport activity and extraction ability.

On this ground, the mode of action of cyclic peptoids **2** and **3** may be rationalized as follows: the build up of the pH-gradient starts a proton–countercation exchange process, rate-limited by the antiport of the countercation, across the phospholipid membrane until the transmembrane proton concentration gradient is eliminated. Cyclic peptoids accelerate the process by promoting the transport of the cation *via* a carrier mechanism and showing cation size-dependent selectivity. This behavior is similar to that observed in analogous conditions for valinomycin, a well known K^+-carrier,[14] although with lower efficiency and cation selectivity.

In conclusion, the synthesis, structural features, alkali metal ion binding and membrane transport of new lipophilic cyclic α-peptoids were disclosed. These results are the first indication that cyclic peptoids can represent new motifs on which to base artificial ionophoric antibiotics. Investigations on the antimicrobial properties of structurally diverse cationic cyclopeptoids are currently underway.

Acknowledgements

Financial support from the University of Salerno, MIUR (contract 2006039071) and Regione FVG is gratefully acknowledged. The authors thank Dr C. Gaeta (University of Salerno) for a valuable discussion.

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