Cd$^{2+}$ coordination: an efficient structuring switch for polypeptide polymers

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1. X-Ray data CBMC-NCA

Crystal data and structure refinement

Identification code: CBMN-NCA
Empirical formula: C_{13}H_{13}N_{6}O_{5}S
Formula weight: 295.30
Temperature: 100(2) K
Wavelength: 0.71073 Å
Crystal system, space group: Orthorhombic, P 2_1 2_1 2_1
Unit cell dimensions:
  a = 4.9869(2) Å  alpha = 90 deg.
  b = 11.7644(5) Å  beta = 90 deg.
  c = 22.1329(8) Å  gamma = 90 deg.
Volume: 1298.49(9) Å³
Z, Calculated density: 4, 1.511 Mg/m³
Absorption coefficient: 0.269 mm⁻¹
F(000): 616
Crystal size: 0.15 x 0.08 x 0.02 mm
Theta range for data collection: 1.96 to 26.36 deg.
Limiting indices: -6<=h<=6, -14<=k<=14, -22<=l<=27
Reflections collected / unique: 29132 / 2649 [R(int) = 0.0401]
Completeness to theta = 26.36: 99.9%
Absorption correction: Semi-empirical from equivalents
Max. and min. transmission: 0.978 and 0.912
Refinement method: Full-matrix least-squares on F²
Data / restraints / parameters: 2649 / 0 / 181
Goodness-of-fit on F²: 1.128
Final R indices [I>2sigma(I)]: R1 = 0.0248, wR2 = 0.0683
R indices (all data): R1 = 0.0280, wR2 = 0.0696
Largest diff. peak and hole: 0.202 and -0.201 e Å⁻³
1. Polypeptides characterizations:

Figure S1. $^1$H-NMR spectra of the polypeptides 1-4 in D$_2$O.

Table S1. Summary of the different characterizations for polypeptides 1-4: upon ROP stands for either PBLG or PCBMC.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Theoretical Dp</th>
<th>Dp upon ROP from $^1$H NMR</th>
<th>Mn upon ROP from SEC (Dp)</th>
<th>PDI upon ROP from SEC</th>
<th>Dp upon deprotection from $^1$H NMR</th>
<th>Mn upon deprotection from SEC (Dp)</th>
<th>PDI upon deprotection from SEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGA 1</td>
<td>30</td>
<td>28</td>
<td>3700</td>
<td>1.23</td>
<td>27</td>
<td>4500</td>
<td>1.15</td>
</tr>
<tr>
<td>PCMC 2</td>
<td>30</td>
<td>27</td>
<td>ND</td>
<td>ND</td>
<td>28</td>
<td>5200</td>
<td>1.24</td>
</tr>
<tr>
<td>PGA 3</td>
<td>60</td>
<td>55</td>
<td>7300</td>
<td>1.21</td>
<td>51</td>
<td>8800</td>
<td>1.19</td>
</tr>
<tr>
<td>PCMC 4</td>
<td>60</td>
<td>61</td>
<td>ND</td>
<td>ND</td>
<td>59</td>
<td>9800</td>
<td>1.27</td>
</tr>
</tbody>
</table>

Dp: polymerization degree; PDI: polydispersity index.
**Figure S2.** FTIR spectra of PGA 1 (left, in grey with DCI, in black with Cd^{2+}) and of PCMC 2 (right, in grey with DCI, in black with Cd^{2+}).

**Figure S3.** $^{113}$Cd NMR spectra used to evaluate the affinity constant of Cd$^{2+}$ towards PGA 1. Solvent: D2O (600 μL) containing 20 mg of sodium acetate (buffer).
**Figure S4.** $^{113}\text{Cd}$ NMR spectra used to evaluate the affinity constant of Cd$^{2+}$ towards PCMC 2. Solvent: D$_2$O (600 μL) containing 20 mg of sodium acetate (buffer). Cd$^{2+}$ exchange rate was found lower with PCMC 2 as compared to PGA 1 certainly because thioether is involved in the coordination sphere of the metal.
Figure S5. $^1$H NMR spectra of PCMC 2 before and after oxidation to sulfoxide (left). FTIR spectra of PCMC 2 after oxidation to sulfoxide (1) and to sulfone (2).

Figure S6. $^{113}$Cd NMR spectra used to evaluate the affinity constant of Cd$^{2+}$ towards PCMC 2 upon oxidation to sulfoxide. Solvent: D$_2$O (600 µL) containing 20 mg of sodium acetate (buffer).

Figure S7. 20 mg/mL solution of PCMC 2 in D$_2$O upon coordination to Cd$^{2+}$ (0.5 equivalent) (right, before oxidation and left after oxidation). This concentration is about 1000 times more concentrated than the concentration used for CD analysis. Before oxidation (right tube), almost 70% of the mass can be recovered by centrifugation. Everything becomes soluble if one adds sodium acetate in excess amount (conditions used for $^{113}$Cd NMR). 30 stands for the theoretical Dp.
3. Preparation of samples and solutions for CD measurements:

• Preparation of mother solutions used for the CD experiments:

PGA I mother solution: The mother solution of the polymer was prepared by dissolving the desired quantity of the polymer in Milli-Q water (polymer concentration: 10 mM in monomer units). For each experiment, an aliquot of the desired volume was taken off from this solution and diluted to the final concentration of 100 μM in monomer units.

PCMC 2 mother solution: The mother solution of the polymer was prepared by dissolving the desired quantity of the polymer in Milli-Q water (polymer concentration: 10 mM in monomer units). For each experiment, an aliquot of the desired volume was taken off from this solution and diluted to the final concentration of 100 μM in monomer units.

CdSO$_4$ mother solution: a solution of CdSO$_4$ 10 mM was prepared by diluting the desired quantity of CdSO$_4$ in Milli-Q water. For each experiment, an aliquot of the desired volume was taken off from this solution and diluted to obtain the final concentration of 50 μM (for 0.5 equivalent per polymer side chain, C$_M$/C$_P$ = 0.5).

ZnSO$_4$ mother solution: a solution of ZnSO$_4$ 10 mM was prepared by diluting the desired quantity of ZnSO$_4$ in Milli-Q water. For each experiment, an aliquot of the desired volume was taken off from this solution and diluted in order to keep the final concentration at 100 μM (for 1 equivalent per polymer side chain, C$_M$/C$_P$ = 1).

Ligand mother solution: a solution of ligand 10 mM was prepared in Milli-Q water. For each experiment, an aliquot of the desired volume was taken off from this solution and diluted to the final concentration of 100 μM (NTA) or 50 μM (EDTA).
PCMC sulfoxide or sulfone derivative mother solution: The mother solution of the PCMC 2 oxide derivative was prepared by dissolving the desired quantity of the polymer once oxidized in Milli-Q water (polymer concentration: 10 mM in monomer units) and the pH brought to neutrality (H$_2$SO$_4$ or NaOH). For each experience, an aliquot of the desired volume was taken off from this solution and diluted to the final concentration of 100 μM in monomer units.

- Preparation of solutions analyzed by CD:

  Typical CD solution (metal salt + PGA 1): 10 μL of PGA mother solution (10 mM) was added to a tube, followed by addition of 10 μL (1 equivalent/ polymer carboxylic unit C$_M$/C$_P$ = 1) of a metal salt solution (10 mM), and 100 μL of a 100 mM acetate buffer of Milli-Q water. The volume was completed to 1 mL (final volume) with Milli-Q water. The samples were always prepared the day before the analyses.

  Typical CD solution (metal salt + PCMC 2): 10 μL of PCMC mother solution (10 mM) was added to a tube, followed by addition of 10 μL (1 equivalent/ polymer carboxylic unit C$_M$/C$_P$ = 1) of a metal salt solution (10 mM), and 100 μL of a 100 mM acetate buffer of Milli-Q water. The volume was completed to 1 mL (final volume) with Milli-Q water. The samples were always prepared the day before the analyses.

  Typical CD solution (metal salt + PGA) for the screening of the stoichiometry by CD: 10 μL of a solution of polymer at 10 mM was added to a tube, followed by the desired quantity of a 10 mM solution of CdSO$_4$ (2.5 μL for 0.25 equivalents, 5 μL for 0.5 equivalents, 7.5 μL for 0.75 equivalents, 10 μL for 1 equivalent, then followed by 100 μL of a 100 mM acetate buffer in Milli-Q water and the volume was completed to 1 mL (final volume) with Milli-Q water. The samples were always prepared the day before analyses.
Typical CD solution (Cd$^{2+}$ salt + polymer) for temperature gradients measurements: 10 μL of polymer mother solution (10 mM) was added to a tube, followed by 5 μL (0.5 equivalent/polymer carboxylic unit C$_{M}$/C$_{P}$ = 0.5) of a CdSO$_4$ mother solution (10 mM), and 985 μL of Milli-Q water eventually mixed with few μL of aqueous solutions of NaOH 0.1 M until pH = 7. The volume was completed to 1 mL of final volume with Milli-Q water. The samples were always prepared the day before the analyses. For acidic polymers measurements, the metal salt solution has been replaced by water. The temperature gradient was analyzed over a range of 10 °C to 80 °C with a scan speed of 10 °C / min. Before starting the measurements at each temperature; the sample was maintained at the desired temperature for 10 minutes in order to be sure that the analysis temperature inside the sample was reached.

Typical CD solution (metal salt + polymer + ligand) for competitive ligand induced destructuring: 10 μL of polymer mother solution (10 μM) was added to a tube, followed by addition of 5 μL (0.5 equivalent/polymer carboxylic unit C$_{M}$/C$_{P}$ = 1) of the CdSO$_4$ mother solution (10 mM), then followed by 100 μL of a 100 mM acetate buffer in Milli-Q water and 840 μL of Milli-Q water. Then 10 μL of the mother solution of NTA ligand (or 5 μL of the mother solution of EDTA ligand) was added. The volume was completed to 1mL of final volume with Milli-Q water. The samples were always prepared the day before the analyses.

Typical CD solution (Metal salt + PCMC sulfoxide or sulfone) for oxidation induced destructuring: 10 μL of PCMC oxide mother solution (10 mM) was added to a tube, followed by the addition of 5 μL (0.5 equivalent/polymer carboxylic unit C$_{M}$/C$_{P}$ = 0.5) of a CdSO$_4$ mother solution (10 mM), then followed by 100 μL of a 100 mM acetate buffer in Milli-Q water and 840 μL of Milli-Q water. For acidic polymers measurements, the metal salt solution has been replaced by water. The volume was completed to 1mL of final volume with Milli-Q water. The samples were always prepared the day before the analyses.
4. Circular dichroism data:

*Figure S8.* Influence of the presence of various metal salts on the CD spectra of an aqueous solution of PCMC 2 (top) and PGA 1 (down) at a buffered pH of 6.5 (acetate 10 mM) and at a mixing ratio C_M/C_P=1. [Polymer] = 50 µM in monomer units.
**Figure S9.** Influence of the molecular weight on Cd$^{2+}$ induced structuring of PGA (top) and of PCMC (down). Dp: polymerization degree (cf table S1)

**Figure S10.** CD spectra of PGA 1 (left) or PCMC 2 (right) polypeptides at a buffered pH of 6.5 (acetate 10 mM) mixed with either CdSO$_4$ or CaSO$_4$. Spectra obtained after Ca$^{2+}$ addition evidenced the regular signatures of coil disordered states. In marked contrast, spectra obtained after Cd$^{2+}$ addition evidenced clear secondary structuring. Mixing ratio $C_{M}/CP=1$ for Ca$^{2+}$ and 0.5 for Cd$^{2+}$. [Polymer] = 50 µM in monomer units.
Figure S11. Increasing pH from 4.0 to 6.5 is a destructuring trigger for secondary structures of PGA 1 (dashed-line, 222 nm is a representative wavelength of the α-helix) or of PCMC 2 (plain line, 200 nm is a representative wavelength of the β-sheet) Polypeptides at a buffered pH (acetate 10 mM) and [Polymer] = 50 μM in monomer units.

Figure S12. Increasing $\frac{C_M}{C_P}$ with Cd² at a buffered pH of 6.5 is a structuring trigger for PGA 1 (dashed-line, 222 nm is a representative wavelength of the α-helix) or of PCMC 2 (plain line, 200 nm is a representative wavelength of the β-sheet) [Polymer] = 50 μM in monomer units.
**Figure S13.** Increasing temperature is a not a good destructuring trigger for secondary structures of PGA$_{30}$ (dashed-line, 222 nm is a representative wavelength of the $\alpha$-helix) or of PCMC$_{30}$ (plain line, 200 nm is a representative wavelength of the $\beta$-sheet). Polypeptides at a buffered pH of 6.5 (acetate 10 mM) and coordinated to Cd$^{2+} (C_M/C_P = 0.5)$. [Polymer] = 50 $\mu$M in monomer units.

**Figure S14.** Influence of thioether sulfoxidation/reduction on the CD spectra of an aqueous acetate buffered solution of PCMC 2. A) coil disordered state at pH 6.5; B from C) $\beta$-sheet structuring upon Cd$^{2+}$ coordination (CdSO$_4$ at a mixing ratio CM/CP = 0.5) at pH 6.5 upon reduction of C; B' from C') $\beta$-sheet structuring at pH 4.0 upon reduction of C'; D from C) coil disordered state upon Cd$^{2+}$ coordination (CdSO$_4$ at a mixing ratio CM/CP = 0.5) followed by thioether sulfoxidation (1% H$_2$O$_2$) and at pH 4.0. D from C' presents a similar CD spectrum (data not shown). [Polymer] = 50 $\mu$M in monomer units. Letters correspond to figure 5.
Figure S15. Influence of thioether oxidation to sulfone on the CD spectra of an aqueous acetate buffered solution of PCMC 2 with or without coordination to Cd^{2+} (CdSO_4 at a mixing ratio CM/CP = 0.5).

Figure S16. Influence of thioether oxidation on the CD spectra of an aqueous acetate buffered solution of PCMC 2 at pH = 4.
Figure S17. Influence of thioether oxidation on the CD spectra of an aqueous acetate buffered solution of PCMC 2 with coordination to Cd$^{2+}$ (CdSO$_4$ at a mixing ratio CM/CP = 0.5).