Cd²⁺ coordination: an efficient structuring switch for polypeptide polymers

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1. X-Ray data CBMC-NCA



Crystal data and structure refine	ment
Identification code	CBMN-NCA
Empirical formula	C ₁₃ H ₁₃ N O ₅ S
Formula weight	295.30
Temperature	100(2) K
Wavelength	0.71073 A
Crystal system, space group	Orthorhombic, P 21 21 21
Unit cell dimensions	a = 4.9869(2) A alpha = 90 deg.
	b = 11.7644(5) A beta = 90 deg.
	c = 22.1329(8) A gamma = 90 deg.
Volume	1298.49(9) A ³
Z, Calculated density	4, 1.511 Mg/m ³
Absorption coefficient	0.269 mm ⁻¹
F(000)	616

F(000) Crystal size Theta range for data collection Limiting indices Reflections collected / unique Completeness to theta = 26.36

Absorption correction Max. and min. transmission Refinement method Data / restraints / parameters Goodness-of-fit on F² Final R indices [I>2sigma(I)] R indices (all data) Largest diff. peak and hole Semi-empirical from equivalents 0.978 and 0.912 Full-matrix least-squares on F^2 2649 / 0 / 181 1.128 R1 = 0.0248, wR2 = 0.0683 R1 = 0.0280, wR2 = 0.0696 0.202 and -0.201 e.A⁻³

-6<=h<=6, -14<=k<=14, -22<=l<=27

29132 / 2649 [R(int) = 0.0401]

0.15 x 0.08 x 0.02 mm

1.96 to 26.36 deg.

99.9 %

1. Polypeptides characterizations:



Figure S1. ¹H-NMR spectra of the polypeptides 1-4 in D₂O.

Polymer	Theoretical	Dp upon ROP	Mn upon ROP	PDI upon ROP	Dp upon	Mn upon	PDI upon
	Dp	from	from SEC (Dp)	from SEC	deprotection from	deprotection	deprotection
		¹ H NMR			¹ H NMR	from SEC (Dp)	from SEC
PGA 1	30	28	3700	1.23	27	4500	1.15
PCMC 2	30	27	ND	ND	28	5200	1.24
PGA 3	60	55	7300	1.21	51	8800	1.19
PCMC 4	60	61	ND	ND	59	9800	1.27

Table S1. Summary of the different characterizations for polypeptides 1-4: upon ROP stands for either PBLG or PCBMC.Dp: polymerization degree; PDI: polydispersity index.







Figure S3. ¹¹³Cd NMR spectra used to evaluate the affinity constant of Cd^{2+} towards PGA 1. Solvent: D2O (600 μ L) containing 20 mg of sodium acetate (buffer).



Figure S4. ¹¹³Cd NMR spectra used to evaluate the affinity constant of Cd^{2+} towards PCMC 2. Solvent: D₂O (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. ¹H 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. ¹¹³Cd NMR spectra used to evaluate the affinity constant of Cd^{2+} towards PCMC **2** upon oxidation to sulfoxide. Solvent: D₂O (600 µL) containing 20 mg of sodium acetate (buffer).



Figure S7. 20 mg/mL solution of PCMC **2** in D₂O 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 ¹¹³Cd 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 1 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 μ M 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 μ M 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 μ M (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 μ M (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 μ M (NTA) or 50 μ M (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 oxydized in Milli-Q water (polymer concentration: 10 mM in monomer units) and the pH brought to neutrality (H_2SO_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 μ M in monomer units.

• Preparation of solutions analyzed by CD :

Typical CD solution (metal salt + *PGA 1):* 10 μ L of PGA mother solution (10 mM) was added to a tube, followed by addition of 10 μ L (1 equivalent/ polymer carboxylic unit C_M/C_P = 1) of a metal salt solution (10 mM), and 100 μ L 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 μ L of PCMC mother solution (10 mM) was added to a tube, followed by addition of 10 μ L (1 equivalent/ polymer carboxylic unit $C_M/C_P = 1$) of a metal salt solution (10 mM), and 100 μ L 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 μ L of a solution of polymer at 10 mM was added to a tube, followed by the desired quantity of a 10 mM solution of CdSO₄ (2.5 μ L for 0.25 equivalents, 5 μ L for 0.5 equivalents, 7.5 μ L for 0.75 equivalents, 10 μ L for 1 equivalent, then followed by 100 μ L 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 (Cd²⁺ 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 CdSO₄ 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 CdSO₄ 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 μ L of PCMC oxide mother solution (10 mM) was added to a tube, followed by the addition of 5 μ L (0.5 equivalent/ polymer carboxylic unit C_M/C_P = 0.5) of a CdSO₄ mother solution (10 mM), then followed by 100 μ L of a 100 mM acetate buffer in Milli-Q water and 840 μ L 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 μ M 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 CdSO₄ or CaSO₄. Spectra obtained after Ca²⁺ addition evidenced the regular signatures of coil disordered states. In marked contrast, spectra obtained after Cd²⁺ addition evidenced clear secondary structuring. Mixing ratio $C_M/CP=_1$ for Ca²⁺ and 0.5 for Cd²⁺. [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 wavelenght of the α -helix) or of PCMC 2 (plain line, 200 nm is a representative wavelenght of the β -sheet) Polypeptides at a buffered pH (acetate 10 mM) and [Polymer] = 50 μ M in monomer units.



Figure S12. Increasing C_M/C_P with Cd^2 at a buffered pH of 6.5 is a structuring trigger for PGA 1 (dashed-line, 222 nm is a representative wavelenght of the α -helix) or of PCMC 2 (plain line, 200 nm is a representative wavelenght of the β -sheet) [Polymer] = 50 μ M in monomer units.



Figure S13. Increasing temperature is a not a good destructuring trigger for secondary structures of PGA₃₀ (dashed-line, 222 nm is a representative wavelenght of the α -helix) or of PCMC₃₀ (plain line, 200 nm is a representative wavelenght of the β -sheet). Polypeptides at a buffered pH of 6.5 (acetate 10 mM) and coordinated to Cd²⁺ (C_M/C_P = 0.5). [Polymer] = 50 μ 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**) β -sheet structuring upon Cd²⁺ coordination (CdSO₄ at a mixing ratio CM/CP = 0.5) at pH 6.5 upon reduction of C; **B' from C'**) β -sheet structuring at pH 4.0 upon reduction of C; **D from C**) coil disordered state upon Cd²⁺ coordination (CdSO₄ at a mixing ratio C_M/C_P = 0.5) followed by thioether sulfoxidation (1% H₂O₂) and at pH 4.0. **D from C'** presents a similar CD spectrum (data not shown). [Polymer] = 50 µM 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+} (CdSO₄ 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+} (CdSO₄ at a mixing ratio CM/CP = 0.5).