# Conformational Transition of Acidic Peptides Exposed to Minerals in Suspension

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**Abstract:** Mineral surfaces probably participated in the chemical processes which led to life in the primitive oceans. The ordered conformations of simple acidic peptides exposed to insoluble minerals are described. Alternating poly(Glu–Leu) adopts a random coil conformation in water due to charge repulsion. The polypeptide extracts cations from insoluble crystalline CdS or molybdenum and adopts an ordered conformation. CdS leads to the formation of $\beta$-sheets whereas molybdenum leads to $\alpha$-helices. Peptides with at least 10-amino acids are necessary to exhibit a significative adsorption onto the surface. Under the same conditions, montmorillonite adsorbs the polypeptide but does not induce any conformational change.

**Keywords:** conformation analysis • circular dichroism • mineral surfaces • peptides

## Introduction

Minerals have always been present on the Earth's surface and they were probably involved in several stages of the origin of life. Bernal[1] was the first to propose that molecules adsorbed on clays were essential for the origin of life. One of the first suggestions of the efficiency of clay minerals in prebiotic chemistry was reported by Paecht–Horowitz[2] who obtained 50-mer polyalanine when polymerizing alanyladenylate in the presence of montmorillonite. Short oligopeptides have also been produced on clay surfaces from free amino acids using wetting-drying heating cycles.[3] Short oligopeptides can be elongated to the corresponding 55-mers on the clay mineral illite by feeding the reaction with fresh monomer and activating agent carbonyldiimidazole.[4] Repeated incubation of short oligo-glutamic acid peptides adsorbed on hydroxylapatite or illite with activated monomer leads to the accumulation of oligomers at least 45 units long.[5] Short oligonucleotides can also be elongated on a suitable mineral surface by feeding the reaction with fresh activated nucleotide.[4] Hydrotalcite concentrates glycolaldehyde phosphate from dilute solution and then catalyses condensation of the carbohydrate subunits leading to potentially prebiotic carbohydrate phosphates.[6] More importantly, transition element containing double layer metal hydroxide minerals with similar concentration efficiency produce ribose-2,4-diphosphate, the nucleoside component of p-RNA, selectively and in high yield.[7]

We have previously shown that strictly alternating homochiral hydrophobic/hydrophilic polypeptides form thermostable $\beta$-sheets. Non-alternating sequences form $\alpha$-helices which are thermolabile. For instance, heating samples in which $\alpha$- and $\beta$-structures coexist increases the amount of $\beta$-structure with loss of $\alpha$-helix.[8] Aggregation of alternating sequences to form $\beta$-sheets is possible only with homochiral (all-L or all-D) polypeptides. Racemic alternating poly(D,L-Leu-D,L-Lys) is unable to adopt a $\beta$-structure and remains unordered.[9] When increasing amounts of L-residues were introduced into the racemic alternating polypeptide, the proportion of $\beta$-sheets increased gradually. Only these segments containing six or more homochiral residues aggregated into stable nuclei of optically pure $\beta$-sheets surrounded by the more fragile heterochiral unordered segments.[10, 11]

We have also shown that $(\mathrm{Glu{-}Leu})_{\sim 55}$, an alternating acidic polypeptide adopts different conformations in the presence of different water-soluble metallic cations.[12, 13, 14] Random coil in pure water, $(\mathrm{Glu{-}Leu})_{\sim 55}$ adopts a $\beta$-pleated sheet structure in the presence of 0.5 equivalent per glutamyl residue of $CaCl_2$ and an $\alpha$-helical structure in the presence of 0.15 equivalent of $FeCl_3$.[12, 13]

In the present paper, we examine if alternating peptides can undergo a conformation transition in the presence of a suspension of insoluble salts and if they adsorb on the mineral surfaces. Oligopeptides, $(\mathrm{Glu{-}Leu})_x$ with $x$ values of 2, 5, and 9 and polydisperse $(\mathrm{Glu{-}Leu})_{\sim 55}$ were exposed to crystalline CdS. $(\mathrm{Glu{-}Leu})_{\sim 55}$ was also exposed to molybdenum metal and to montmorillonite clay.

Sulfides were chosen as the solid source in relation with the primitive sulfide metabolism proposed by Wächtershäuser.[15] In our hands, iron sulfide, a naturally abundant phase, was not

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stable enough to allow reproducible UV and CD measurements. Cadmium sulfide which was found easy to handle was chosen as a representative of the sulfide mineral surfaces. Naturally more abundant phases like sulfides of iron, zinc, and copper will be tested to come closer to a natural environment. Molybdenum was chosen as a metal known to interact with sulfur atoms which might have participated in the thioester world promoted by De Duve.[16]

## Results

**(Glu–Leu) peptides and CdS**: An aqueous solution of (Glu–Leu)$_{\sim 55}$ was stirred with different amounts of crystalline hexagonal $\alpha$-CdS (1–3 μm) in suspension. After centrifugation, the supernatant was analysed by circular dichroism. For each CdS concentration, the behavior of the polypeptide was followed as function of time. The polypeptide exhibited a transition from a random coil conformation to a $\beta$-sheet structure. The maximum transition was observed for 100 equivalents of CdS per glutamyl residue and 4 h incubation (Figure 1). In the presence of amounts of CdS larger than 100 equivalents, no peptide remained in solution. Since no separate peptide precipitation could be observed, it is likely that the peptide chains adsorbed onto the mineral surface. For larger incubation times, the amounts of peptide in solution decreased progressively at all CdS concentrations studied (Figure 2). The commercial 1-3 μm CdS grains were ground to increase the mineral surface available for the peptide. Addition of 100 equivalents of fine grained CdS significantly decreased the amount of peptide remaining in solution (Figure 3). Short oligo(Glu–Leu) peptides have also been studied to determine the minimal chain length required for both conformational transition and adsorption. (Glu–Leu)$_{2,5,\text{ and }9}$ become adsorbed totally after 24 hours in the presence of 100 equivalents of CdS. Different behaviours were observed

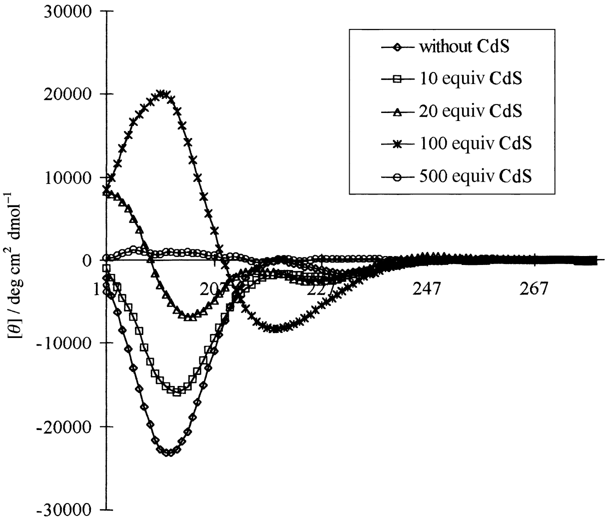

Figure 1. CD spectra of (Glu–Leu)$_{\sim 55}$ after 4 h incubation with various amounts of CdS.

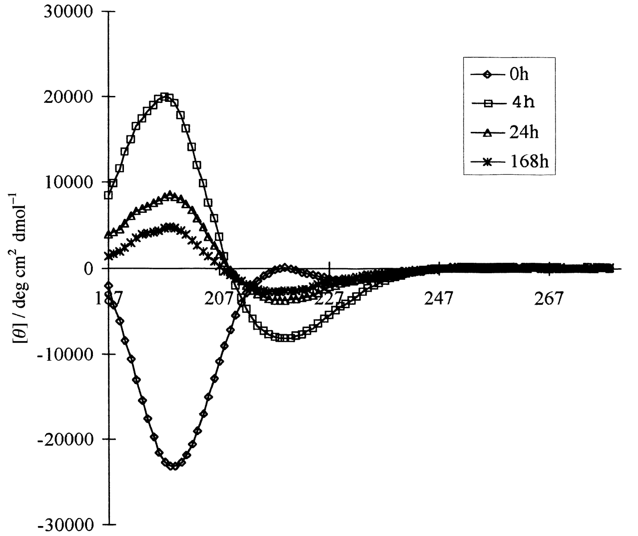

Figure 2. CD spectra of (Glu–Leu)$_{\sim 55}$ in the presence of 100 equivalent of CdS. Evolution with time.

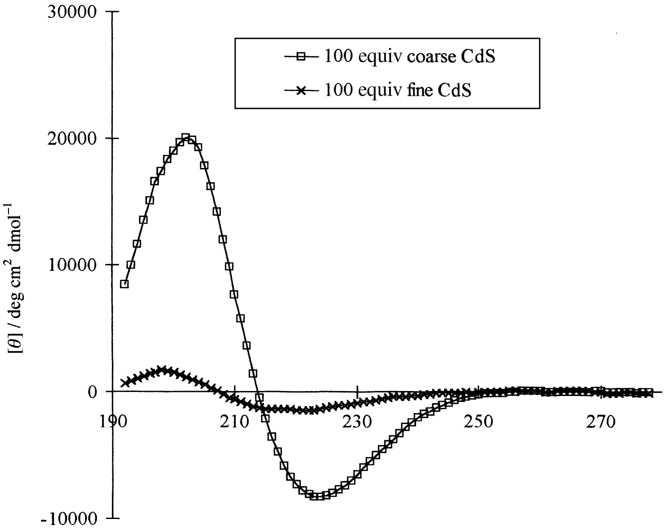

Figure 3. CD spectra of (Glu–Leu)$_{\sim 55}$ after 4 h incubation with two different granulometries of CdS.

**Abstract in French:** *Les surfaces minérales participèrent vraisemblablement aux processus chimiques qui permirent l'apparition de la vie sur Terre. Les conformations adoptées par des peptides acides simples en présence de surfaces minérales insolubles sont décrites. Le peptide alterné poly(Glu–Leu) adopte une conformation désordonnée dans l'eau en raison des répulsions électrostatiques. Le polypeptide arrache des cations de sulfure de cadmium cristallin insoluble ou du molybdène métallique et adopte alors une structure ordonnée, feuillets β avec CdS et hélice α avec le molybdène. Il faut au moins 10 acides aminés dans la chaîne peptidique pour que le peptide s'adsorbe sur la surface minérale. Dans les mêmes conditions, la montmorillonite adsorbe le peptide mais n'induit aucune structure ordonnée.*

when the amounts of CdS were increased from 1 to 10 equivalents. (Glu–Leu)$_9$ was strongly adsorbed while (Glu–Leu)$_2$ was practically not sensitive to the presence of CdS. (Glu–Leu)$_5$ showed an intermediary behaviour. No marked transition to $\beta$-sheet structure have been observed with these short peptides (Figure 4).

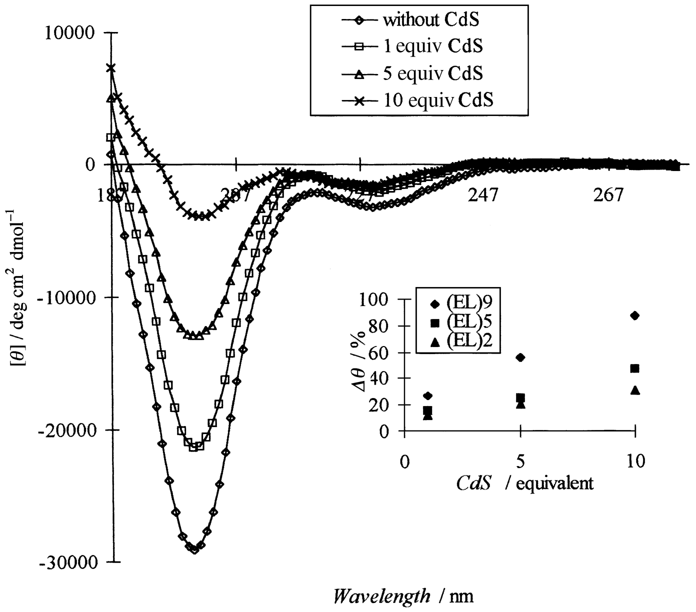

Figure 4. CD spectra of (Glu–Leu)$_9$ after 24 h incubation with various amounts of CdS. Insert: variation of ellipticities at 199 nm $\Delta[\theta] = ([\theta]_{H_2O} - [\theta]_{CdS})/[\theta]_{H_2O}$ for (Glu–Leu)$_2$, (Glu–Leu)$_5$, and (Glu–Leu)$_9$ after 24 h incubation with amounts of CdS increasing from 0 to 10 equivalents.

**Poly(Glu–Leu) and molybdenum and montmorillonite**: An aqueous solution of (Glu–Leu)$_{\sim 55}$ was stirred with different amounts of molydenum metal in suspension. For each molybdenum concentration, the behavior of the polypeptide was followed as function of time. The polypeptide exhibited a transition from a random conformation to an $\alpha$-helical structure. The maximum transition was observed for 50 equivalents of molybdenum and 4 h incubation (Figure 5). In the presence of larger amounts of molybdenum, the polypeptide becomes adsorbed on the metallic surface. For larger incubation times, the polypeptide was adsorbed when incubated with more than 10 equivalents of molybdenum.

The aqueous solution of (Glu–Leu)$_{\sim 55}$ was also stirred with increasing amounts of montmorillonite clay in suspension. When less than 10.9 mg of clay per mmole of glutamyl residue were present, the peptide remained in solution as random coil. For larger amounts of clay, the peptide becomes adsorbed.

## Discussion

In pure water, the sodium salt of (Glu–Leu)$_{\sim 55}$ adopts a random coil conformation due to charge repulsions. We have previously shown that very small amounts of soluble polyvalent metallic cations, typically 0.5 equivalent per acidic residue for a divalent cation, are able to screen the side chain charges thus allowing the formation of water soluble ordered conformations. In the present study, we show that acidic peptides can extract the cations required for charge screening from insoluble crystalline CdS or Mo. The solubility at pH 7 of crystalline $\alpha$-CdS is $2.16 \times 10^{-7}$ g in 100 mL water[17] corresponding to a $1.5 \times 10^{-10}$ M CdS concentration due to the following reaction:

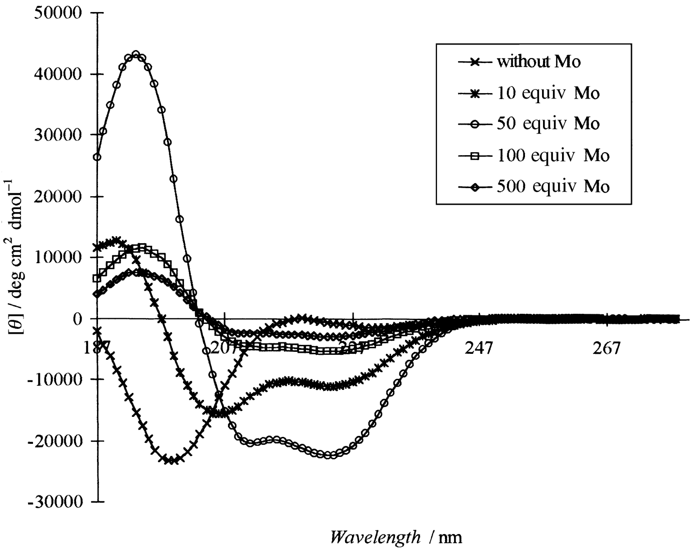

Figure 5. CD spectra of (Glu–Leu)$_{\sim 55}$ after 4 h incubation with various amounts of Mo.

$$CdS + H_2O \longrightarrow CdOH^+ + HS^-$$

Since concentrations of $8.3 \times 10^{-4}$ M in glutamyl residues were used for the peptide, the glutamyl residues were about $5 \times 10^6$ more abundant that the cadmium cations in solution. Obviously, the peptides favor the formation of cadmium cations which allow the coil to $\beta$-transition. The same remark holds for the molybdenum grains, the surface of which are oxidized in water. In water molybdenum metal, slowly transforms into molybdenum hydrous oxide species[18] with oxidation states about 5 and 6, like $[Mo_2O_4(H_2O)_6]^{2+}$. These ions induce the formation of an $\alpha$-helix. Helical conformations have already been obtained with soluble $Fe^{3+}$ ions which interact both with the backbone carbonyl and the glutamyl side chain carboxylic groups.[13] Once having extracted cations from the mineral surface the peptides adopt a regular structure. When offering larger mineral surfaces to the peptides, they adsorb onto the surface. The process is chain-length dependent. At least, a 10-amino acid long peptide chain is necessary to exhibit significant adsorption. Under the same conditions, montmorillonite adsorbs the polypeptide but does not induce any conformational transition.

Our results suggest that the dissolved peptides are complexed and ordered by metal ion species extracted from the insoluble minerals. The next step will consist to study what happens to the conformation of the peptides once they have been adsorbed on the mineral surface.

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## Experimental Section

The synthesis of polydisperse (Glu–Leu)$_{\sim 55}$, (Glu–Leu)$_9$, (Glu–Leu)$_5$, and (Glu–Leu)$_2$ have been described in a previous paper.[12]

The acidic form of poly(Glu–Leu) is insoluble in water. The dry free carboxylic form dissolves slowly when suspended in water in the presence of the exact amount of 0.1M sodium hydroxide required to neutralize the acidic groups of the polypeptide. Addition of mineral surfaces was achieved by adding the required amounts (equivalent per mole of glutamyl residues or mg of mineral surface). CdS and Mo used (1-3 µm) were purchased from Alpha, montmorillonite clay was kindly provided by J. P. Ferris from the Rensselaer Polytechnic Institute, Troy, N.Y.

The pH was ajusted to 7 in each sample. The peptidic solutions concentrations were 0.82mM in Glu for all experiments. Concentrations of polymer solutions were determined from the optical density at 205 nm, assuming an extinction coefficient of 3300 $\mathrm{L\,mol^{-1}\,cm^{-1}}$ per mean residue for the unordered conformation. Peptide solutions were stirred during 4, 24, or 168 h at 25 °C and centrifuged before use. The supernatants were studied by circular dichroism. CD spectra were recorded between 185 and 280 nm on a Jobin–Yvon Mark IV dichrograph. CD spectra of random coil conformations are characterized by a negative contribution at about 197 nm. $\beta$-Sheets exhibit a trough near 217 nm and a positive contribution near 197 nm. $\alpha$-Helices show two negative contributions near 208 and 222 nm.

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