The effect of calcium ions on compressibility of amphiphilic peptides at air water interface

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Peptides composed of alternating hydrophobic and hydrophilic residues may preferably reside at interfaces between hydrophobic and hydrophilic phases. Structural studies on amphiphilic β-sheet peptides have been conducted both with synthetically designed systems and in context of natural proteins.[1-2] The peptide Pro-Asp-(Phe-Asp)$_5$-Pro denoted P_{FD}-5, has been previously shown to form two-dimensional β-sheet assemblies at air-water interface.[3] Monolayers of the peptide were used as a template to mineralization of calcium salts including hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) that is the major inorganic component of bone and teeth.[4] P_{FD}-5 was also shown to form hydrogels with calcium ions as efficient cross linkers between the peptides' strands.[5] Both at interfaces and in hydrogels the peptides assume the β-sheet structure therefore. Hence grazing incidence X-ray diffraction (GIXD) studies applied to peptide monolayers at interfaces can provide insight on the effect calcium ions may have on the peptide self-assembly properties.

Here we utilized GIXD along Langmuir isotherms to characterize the monolayer of P_{FD}-5 in the presence of 0.2M $CaCl_2$ in Tris buffer solution (pH~7.3). Bragg peaks were utilized to evaluate the compressibility of the crystalline monolayer that manifests in contraction of the smallest detected spacing, assumed the $d_{(0,1)}$ spacing. The crystalline compressibility, $C_C = -(\partial \ln d_{0,1} / \partial \pi)$ was evaluated both for peptide film on water and compared with compressibility of the peptide on calcium chloride solution.

The surface pressure vs. the mean molecular area isotherm of the peptide on water (Fig. 1a) exhibits a limiting area per molecule of ~210Å^2 which matches the peptide strand dimensions (~45 x ~4.7Å, strand length and hydrogen bond spacing, respectively). The limiting area per molecule of the peptide on the buffered calcium solution was found to be smaller (~100Å^2) indicating partial dissolution of the peptide in the more basic subphase of the buffer. According to GIXD measurements, the long axis spacing $d_{(0,1)}$, of the P_{FD}-5 monolayer on water was measured to be ~46Å, matching the length of the peptide and in agreement with previously reported GIXD measurements of the same peptide.[6] The peptide also exhibits 10.9 m/N crystalline compressibility, slightly larger than the 8.7 m/N, previously reported for the peptide.[6] Crystalline compressibility that manifests in d(0,1) contraction along the macroscopic compression of the film, has been previously attributed to peptide backbone bending out of the water surface.

P_{FD}-5 monolayer on the calcium solution behave quite differently with an initial $d_{(0,1)}$ spacing of ~57Å that is significantly larger than the length of the peptide, hence pointing to a unit cell which is composed of two peptide strands along the (0,1) direction. Spacing of ~60 Å was previously reported for P_{FD}-5 monolayer but on a film that underwent compression and expansion. Nevertheless, it is possible that the ~60 Å represents a preferred mode of a close packing that on calcium maybe achieved due to the screening effect of more specific interactions with the ions. This spacing implies a unit cell composed of two peptides along the long backbone axes that may extend to ~ 45 x 2 = 90 Å in length. Various factors and combinations thereof may account for the large difference between the observed spacing and this theoretically estimated length. The assembly could be significantly distorted compared to the unit cell of the peptide on water (~ 45 x 4.7 Å dimensions in a rectangular unit cell). The unit cell could be with an angle < 90° (between *a* and *b* axes) that would result in a spacing shorter than the estimated length of two peptides. In addition, the β-sheet backbones may also be slightly bent out- or within- the plane of the air-water solution interface, because of interactions with the calcium ions (Fig. 2). The lattice formed over calcium solution, appeared to be much more sensitive to compression based on the compressibility values C_C = 61.1m/N at low surface pressure and 21.1m/N, calculated for the curves of π versus ln $d_{(0,1)}$

(Fig. 1b). In summary based on Langmuir isotherms and GIXD measurements P_{FD}-5 interacts with calcium ions in a manner that changes its crystalline packing and compressibility.

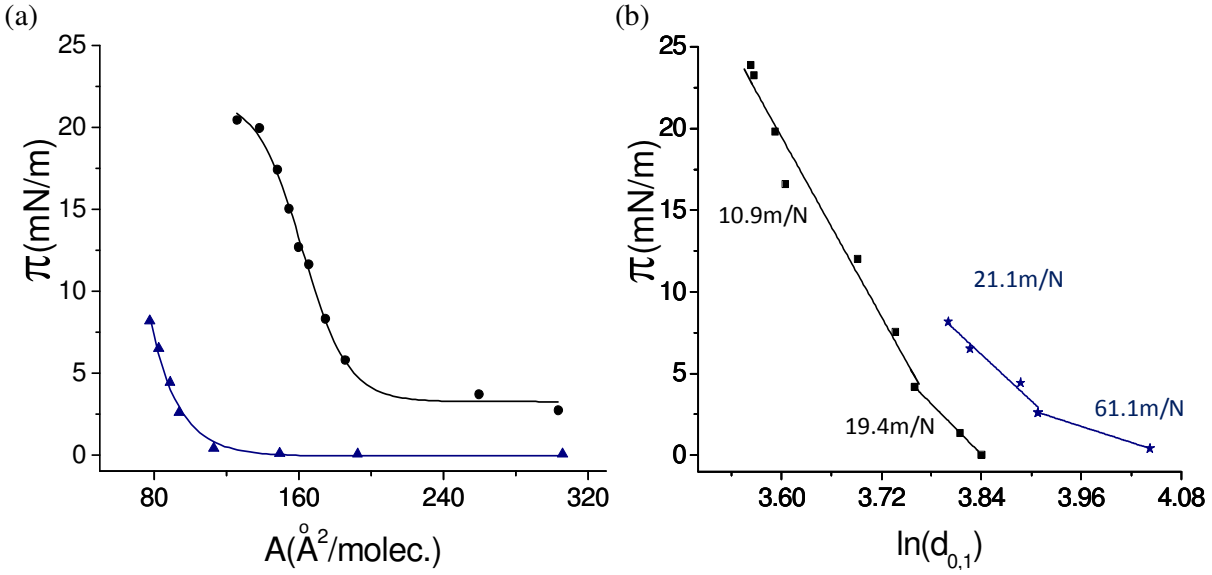

Figure 1: a) Langmuir surface pressure-area isotherm curves of P_{FD}-5 on water (●) and on calcium solution (▲). GIXD measurements were acquired at the marked points along the isotherm; b) ln $d_{(0,1)}$ versus surface pressure of P_{FD}-5 on water (■) and on calcium solution (*). Crystalline compressibility values were assigned for each linear part of the curve.

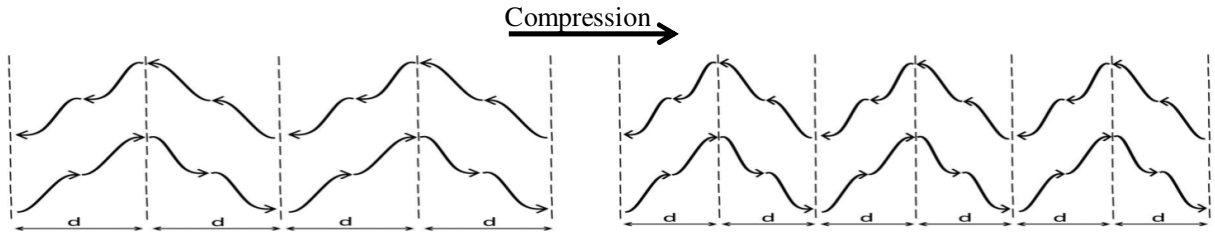

Figure 2: Schematic representation of β-sheet backbone in buffered solution of calcium ions under compression. The dashed line represents the unit cell $d_{(0,1)}$ spacing.

References

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