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6. AUTHOR(S)
Gregory N. Tew
Lachelle Arnt

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Massachusetts, Amherst
408 Goodell, 140 Hicks Way
Amherst, MA 01003-9272

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13. ABSTRACT (Maximum 200 words)

Structural proteinomics is providing rapid insight into the structural and biological functions of many proteins. Our intention was to use this understanding to develop polymers that are more stable and inexpensive to produce than natural proteins, but nevertheless mimic their important biological properties. Toward this goal, we have focused on the host defense peptides, which are a broad class of peptides with remarkable antimicrobial properties against a host of organisms including Gram-negative and Gram-positive bacteria. These peptides have a common physiochemical motif that leads to their biological activity, which is an overall cationic and amphiphilic architecture. We developed polymers that also have an overall cationic and amphiphilic architecture similar to the peptides. Recently, similar work has been reported on beta-amino acids however these materials are still very labor intensive and expensive to produce. Nonetheless, they demonstrate that non-natural polymers can be used to mimic peptide activity. We developed a family of very inexpensive polymers that mimic the structural characteristics of amphiphilic beta-sheet peptides.

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Sincerely,

Enclosure 3

(1) Foreword (optional): not provided

(2) Table of Contents (if report is more than 10 pages): less than 10 pages

(3) List of Appendixes, Illustrations and Tables (if applicable): Figures included in text

(4) Statement of the problem studied:

Structural proteomics is providing rapid insight into the structural and biological functions of many proteins. Our intention was to use this understanding to develop polymers that are more stable and inexpensive to produce than natural proteins, but nevertheless mimic their important biological properties. Toward this goal, we have focused on the host defense peptides, which are a broad class of peptides with remarkable antimicrobial properties against a host of organisms including Gram-negative and Gram-positive bacteria. These peptides have a common physiochemical motif that leads to their biological activity, which is an overall cationic and amphiphilic architecture. We developed a family of very inexpensive polymers that mimic the structural characteristics of amphiphilic beta-sheet peptides.

(5) Summary of the most important results:

The design of secondary conformation and chemically rich polymers that mimic natural biopolymers in function and structure is an important goal. Polymers based on *meta*-phenylene ethynylenes were patterned with polar, cationic and non-polar alkyl groups along the backbone with a repeat similar to amphiphilic β-sheet peptides. The general polymer structure used in this study is shown in Figure 1 and was designed with different length alkyl groups to probe the structure as well as their membrane activity.[1,2] Polymers **1** (pentoxy), **2** (octoxy), and **3** (dodecoxy) were analyzed at the air-water interface using a Langmuir trough and the pressure-area isotherms are shown in Figure 2. By extrapolating the steepest slope of the isotherm of **1** to zero pressure, an area per repeat unit of ~41 Å^2 is obtained. The areas per repeat unit for polymers **2** and **3** are ~42 Å^2 and ~45 Å^2, respectively. Films of these two polymers do not collapse at pressures up to ~48 mN/m which likely results from the increased length of the alkyl side chain. These areas per repeat unit are comparable to that of **1**, suggesting all three amphiphilic polymers adopt edge-on structures similar to that presented in Figure 3. The slope of all three curves is less steep than observed for many rigid amphiphilic polymers[3] but similar to those observed by Swager and co-workers in systems with conformational

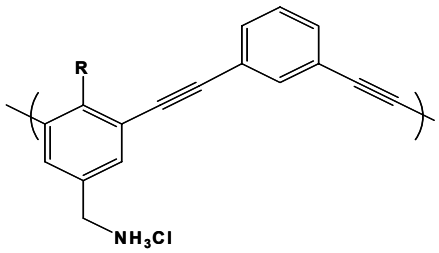

Figure 1. Structures of the polymers used in this study.

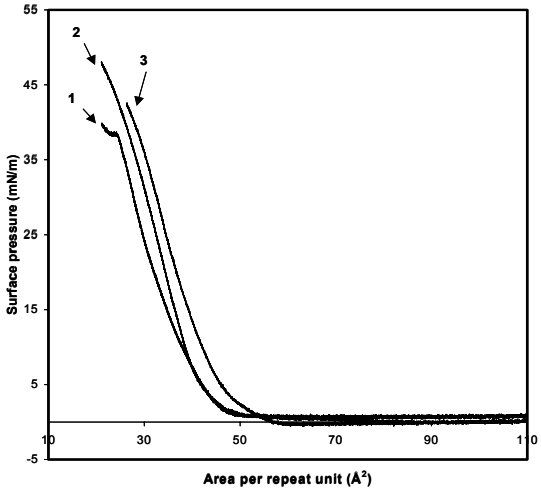

Figure 2. Langmuir films of polymers used in this study.

flexibility.[4] These polymers likely adopt a random coil conformation at the interface as opposed to a more aggregated arrangement of chains before compression. In fact, fitting the data to the equation of state, $\pi(A-A_0)= kT/nDP$, where π is the observed surface pressure for a given area per repeat unit (A), DP is the average number of repeat units per molecule, k is Boltzmann's constant, and T is the temperature.[5] The data was analyzed between surface pressures of 1.4 mN/m and 3.0 mN/m. Linear regression gives A_0, the limiting molecular area, and n, the degree of association, which should be near unity for an ideal gas. The values of A_o show good agreement with those obtained by simple extrapolation of the pressure-area isotherms to zero pressure. Also, the degree of polymer chain aggregation is very small or non-existent before compression which suggests the polymers form a two dimensional gas-like state at high dilution.

The extrapolated area per repeat unit at zero pressure and A_o for the three polymers is consistent with an edge-on orientation as shown in Figure 3 which was developed based on both geometric and molecular dynamics calculations. The length of the repeat unit in an all-*trans* conformation is 11 Å and the π-π distance between chains was assumed to be 4 Å[4] resulting in an area per repeat of 44 Å^2. Similarly, dynamics calculations performed with Materials Studio on two oligomers of four repeats each provided an area of 44.7 Å^2 for polymer **1**. Therefore, the edge-on model shown in Figure 3 is most consistent with the experimental data. Other orientations with one or both aromatic rings parallel to the interface would produce areas on the order of ~140 and 200 Å^2, respectively.

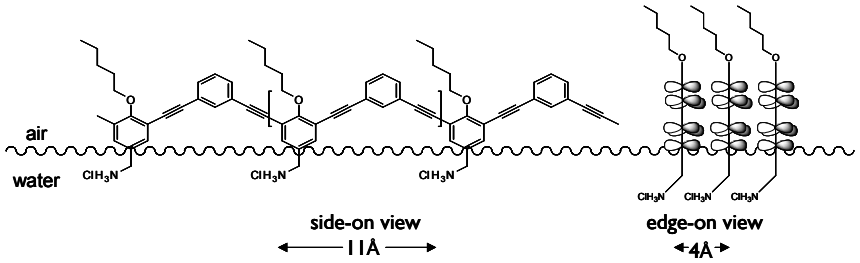

Figure 3. Left: Side view of single chain of amphiphilic polymer **1** at the air-water interface seen from the side. Right: Schematic end view of three adjacent π-stacking polymer chains.

The end-on view in Figure 3 shows the aromatic rings directly perpendicular to the air-water interface at a separation distance of 4 Å. It is known that PPE molecules prefer to pack at a slightly off-set angle as opposed to directly on top of each other. At the air-water interface, this slip stacked orientation can occur through two different mechanisms. Either the aromatic rings can adopt a slightly non-perpendicular angle with the air-water interface (not shown) or by adopting a right angle but sliding one chain along the water surface, with respect to the neighboring chain, into or out of the plane of the page (shown). The latter orientation is most likely here since it occupies the smallest area, although no direct experimental evidence relating to these details for either orientation has yet been obtained.

The structure of this monolayer draws comparison to β-sheet peptides as a result of the patterning of P and NP groups onto opposite faces of the structure to produce amphiphilic plates. Both these polymers and β-sheet peptides have P and NP groups repeating with a pattern of two along the backbone; however, β-sheets are held together by hydrogen bonds oriented parallel to the air-water interface which are replaced in these systems with π-π interactions between adjacent polymer backbones (Figure 2). The typical distance

between backbones in β-sheets is 4.7 Å[6] compared to approximately 4.0 Å for these polymers.

During these experiments, we observed β-sheet like structure at the air-water interface, which prompted further investigation into solid-state structures of these polymers. When the X-ray diffraction patterns of the particles prepared by precipitation were analyzed, the data fit a bilayer structure where the polymers were packed so that in one direction there is π-stacking and in the other direction there are interactions between the hydrophilic or hydrophobic sides of two chains. The cationic charges are proposed to have a slight offset from each other in order to minimize free volume and establish favorable interaction. The curves are shown in Figure 4 for polymers **1** and **3** and the reflections correspond to d-spacings of 23.17 and 31.22 Å, respectively for the 001 peak. In the curve for **1**, a peak with d-spacing equal to at 12.02 Å can be seen but it is unclear at this time whether this is an 002 or 010 peak related to the polymer's chemical repeat. This data agrees with a β-sheet like structure obtained from precipitation from a poor solvent. This is particularly interesting since usually polymers precipitated in this manner form amorphous structures.[1]

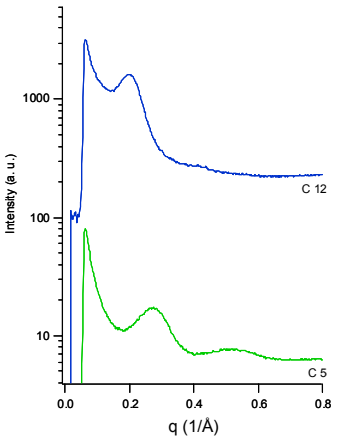

Figure 4. SAXS of **1** and **3**.

The conformation of facially amphiphilic polymers both at the air-water interface and in the solid-state was studied. The ability to characterize this β-sheet like structure in a polymer system and control the intramolecular distances by the length of the alkyl side chain provides great insight into this system. The importance of facially amphiphilic design is confirmed by the fact that these polymers should have ordered precipitates unlike conventional polymers. Further investigation of these polymers is currently underway.

(6) Listing of all publications and technical reports supported under this grant or contract. Provide the list with the following breakout, and in standard format showing authors, title, journal, issue, and date.

(a) Papers published in peer-reviewed journals:

Arnt, L.; Tew, G. N. *Langmuir* **2003**, ASAP article Feb 14th.

(b) Papers published in non-peer-reviewed journals or in conference proceedings:

Arnt, L.; Tew, G. N. *ACS-Polymer Div. Spring 2003.*

Tew, G. N. *ACS-Polymer Div. Spring 2003.*

(c) Papers presented at meetings, but not published in conference proceedings

none

(d) Manuscripts submitted, but not published

none

(e) Technical reports submitted to ARO

none

(7) List of all participating scientific personnel showing any advanced degrees earned by them while employed on the project:

Lachelle Arnt

(8) Report of Inventions (by title only): None

(9) Bibliography

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(10) Appendixes: None