Determination of the Extinction Coefficient for *N*-Substituted Glycines Using Ultra Violet Spectroscopy

Polypeptoids may provide many medical benefits such as serving as additives to a lung surfactant replacement for prematurely born infants with respiratory distress. Polypeptoids exhibit significant conformational stability and are resistant to proteases. Studies using a pulsating bubble surfactometer suggest that the use of polypeptoids in a lung surfactant replacement is promising. This research was aimed at improving understanding of the spectroscopic properties of short peptoid oligomers. In particular, we attempted to determine the molar extinction coefficients, **ε**, for various polypeptoids. By determining their extinction coefficient, we hoped to be able to calculate the exact molar concentration of polypeptoids in organic and aqueous solutions. Scientists producing polypeptoids are working to harness as many of the advantageous properties of a polypeptide as possible. Presently, the concentrations are determined by taking careful mass measurements, but this process is time-consuming and prone to error. In order to find the extinction coefficient we used Ultra-Violet (UV) Spectroscopy to measure the light absorption of peptoid samples at a known concentration (as determined by careful mass measurements). The graph of **ε** vs the molar concentration of a polypeptide was found to be linear, while previous, preliminary studies had shown the relationship to be non-linear. In particular, the previous studies had indicated a possible ''hypochromic'' effect (similar to what is seen for double stranded DNA solutions in water) as the cause for the deviation from linearity. We believe that π-π stacking intermolecular activity in polypeptoids with aromatic side chains may be the cause of the shift in the wavelength of maximum absorbance.

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Introduction

Peptoids differ from peptides only in the appendage of the side chains. The side chains in a peptoid are attached to the amide nitrogen as opposed to the α-carbon (Figure 1). Peptoids have achiral backbones, and those shown to be helical in structure have two-thirds α-chiral bulky aromatic side chains. The need for stable and non-immunogenic proteins has prompted scientists to develop various classes of synthetic proteins, among which are vinylogous polypeptides, peptide nucleic acids, oligoureas, olligopyrrolinones, β-peptides, γ-peptides, and polypeptoids. Peptoids are particularly desirable because of their ability to form stable helices, their resistance to proteases, and their lack of an immunogenic reaction. Previous studies found that helices are formed because the side chains are attempting to reduce steric clashes with bulky parts of the peptoid backbone. Additionally, several studies were conducted to test the conformational stability of the peptoid helices. Together, these studies determined guidelines for a stable helix and the effects of chain length and sequence on secondary structure. Helices are most stable when 50% of the sequence is composed of α-chiral aromatic side chains and when submonomers containing α-chiral aromatic side chains are put on the carboxy amide terminus. Further studies showed chains longer than 11–13 monomers cease to show an increase in the helicity of the peptoid. This does not mean however, that the peptoid ceases to form a helix at these particular wavelengths.

The many possible medical benefits of peptoids led to several studies, including the antimicrobial properties of peptoids (magainin-2 amide), but chiefly the use of peptoids in a lung surfactant (LS) replacement. LS is necessary to reduce the surface tension on the alveoli surface. Premature babies who are born with respiratory distress syndrome (RDS) lack LS. In the past, LS was animal derived, which raised concerns about the purity and the immunogenic effect in the human body. Proteins SP-B and SP-C are present in LS and necessary for LS to function properly. They are both helical, hydrophobic, amphipathic proteins and easily mimicked. Studies using a pulsating bubble sufactometer (PBS) show that the peptoids of SP-C do in fact display the same biophysical properties of biological SP-C. Taking into account the vast potential of peptoids, it is necessary to be able to accurately calculate concentrations. To do so, we attempted to find the extinction coefficient of various peptoids using Ultra Violet (UV) Spectroscopy. In this study we used the following peptoid sequences $Nspe_9$, $Nspe_{12}$, $Nile_{15}$, H-(*N*Lys-*N*spe-*N*spe)$_5$–NH_2, H-(*N*Lys-*N*spe-*N*spe)$_2$-NH_2, and H-(*N*Lys-*N*rsb-*N*rpe)$_4$-NH_2. *N*spe$_9$ and *N*spe$_{12}$ have all aromatic side chains, H-(*N*Lys-*N*spe-*N*spe)$_5$–NH_2, H-(*N*Lys-*N*spe-*N*spe)$_2$-NH_2, and H-(*N*Lys-*N*rsb-*N*rpe)$_4$-NH_2 have two-thirds aromatic side chains, and $Nile_{15}$ has no aromatic side chains. We hypothesized that π-π intermolecular activity between the aromatic side chains in peptoids *N*spe$_9$, *N*spe$_{12}$, H-(*N*Lys-*N*spe-*N*spe)$_5$–NH_2, H-(*N*Lys-*N*spe-*N*spe)$_2$-NH_2, and H-(*N*Lys-*N*rsb-*N*rpe)$_4$-NH_2 are producing a hypochromic effect at low concentrations.

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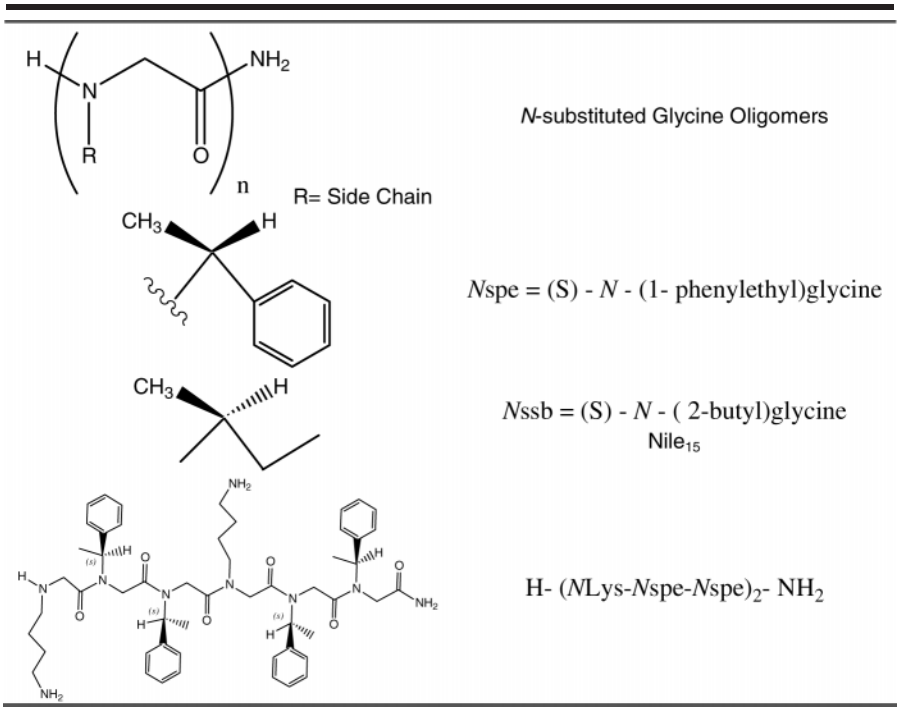

Fig 1. Structural characteristics of peptoids

METHODS

All peptoids were dissolved in ~2mg/mL solutions. $Nile_{15}$ was dissolved in acetonitrile (ACN). $Nspe_9$ and 12 were dissolved in acetonitrile and methanol (MeOH). Peptoids H-(*N*Lys-*N*spe-*N*spe)$_5$-NH_2, H-(*N*Lys-*N*spe-*N*spe)$_2$-NH2, and H-(*N*Lys-*N*spe-*N*spe)$_4$-NH_2 were dissolved in deionized water. In addition, we acquired a peptide, SP-B2, to serve as a control. SP-B2 was also dissolved in a ~2mg/mL solution of deionized water. Next, the identities of the samples were confirmed using Electrospray Ionization Mass Spectrometry (ESI-MS). Then, we proceeded to prepare samples for UV Spectroscopy. We wanted to have at least eight data points between 1μM and 15μM concentrations because this range was where hypochromicity was suspected. We made solutions at concentrations starting at 60μM then, 50μM, 40μM, 30μM, 25μM, 20μM, 18μM, 16μM, 14μM, 12μM, 10μM, 8μM, 6μM, 4μM, and 2μM. After the samples were prepared UV Spectroscopy was used. A cuvette with a path length of 1 centimeter and a volume of 40μL was used.

All readings were taken at a wavelength of 220 nm or 260 nm because the chromophores on the amide bonds and phenyl rings in the peptoids, respectively, absorb light. $Nspe_9$ and $Nspe_{12}$ were both run in ACN and MeOH at 260 nm and again at 220 nm. All other peptoids were run at 220 nm. Full spectra between 350 and 200 nm was obtained for $Nspe_9$ and $Nspe_{12}$. Individual samples were read three times and each set of samples was run three times. We obtained the extinction coefficient by using Beer's Law, which is expressed as: $A = \varepsilon lc$. In the equation A is absorbance, ε, the molar extinction coefficient in L/microMol$_{centimeters}$, l is the path length of the cuvette in centimeters, and c is the molar concentration. Beer's Law implies that the relationship between absorbance and concentration is linear at low concentrations and the relationship between absorbance and ε is constant. Hence, in order to find ε we rearranged the equation to: $\varepsilon = A/cl$.

RESULTS

We hypothesized that peptoids with non-aromatic side chains would have an extinction coefficient, ε, while peptoids with all or partial aromatic side chains would display a hypochromic effect. The data for $Nspe_9$, $Nspe_{12}$, 1, 2, and 3 at 260 nm all displayed a sudden increase in ε. This sudden increase in ε could indicate a shift in wavelength. Our data for $Nile_{15}$, SP-B2, $Nspe_9$, and $Nspe_{12}$, 1, 2, and 3 at 220 nm follows Beer's Law by demonstrating a linear trend when absorbance is plotted as a function of concentration in all peptoids at 220 nm. Further, when absorbance was graphed as a function of concentration at 260 nm for $Nspe_9$, $Nspe_{12}$, and $Nspe_2$, no linear trend could be observed. Full spectra was preformed for *N*spe9 in ACN. A slight shift in wavelength was evident. Extinction coefficients were calculated for all peptoids at 220 nm.

CONCLUSION

We obtained several peptoids: $Nile_{15}$, $Nspe_9$, $Nspe_{12}$, H-(*N*Lys-*N*spe-*N*spe)$_5$–NH_2, H-(*N*Lys-*N*spe-*N*spe)$_2$-NH_2, and H-(*N*Lys-*N*rsb-*N*rpe)$_4$-NH_2. Thus far, we have come to the following conclusions. First, that when dissolved in ACN and at 260 nm homooligomers of *N*spe produce a hypochromic effect at 260 nm. Peptoids with no aromatic side chains appear to have an extinction coefficient, i.e., ε for $Nile_{15}$ is 0.024 L/μMol$_{centimeters}$. Next, peptoids with two-thirds aromatic side chains may be producing a hypochromic effect at 260 nm. In addition, homooligomers of *N*spe do not produce a hypochromic effect at 220 nm, whether in ACN or MeOH. Their graphs for absorbance versus concentrations appear to be quite linear. This also holds true for H-(*N*Lys-*N*spe-*N*spe)$_5$–NH_2, H-(*N*Lys-*N*spe-*N*spe)$_2$-NH_2, and H-(*N*Lys-*N*rsb-*N*rpe)$_4$-NH_2 at 220 nm.

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