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Advance Development of Guanosine Containing Nucleopeptides

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Abstract

In this article, we describe the creation and characterisation of self-assembling nucleopeptides containing guanosine that can form nanosheets and nanofibers. We suggest that the nucleopeptide's peptide component drives the assembly into -sheet structures, with hydrogen-bonded guanosine generating additional secondary structures cooperatively inside the peptide framework. This is based on spectroscopic and microscopy studies. It's interesting to note that the C-terminal peptide chemistry, rather than the metal cation responsiveness common to guanine-based materials, is what drives the different supramolecular morphologies. This research will aid in the advancement of the design of applications for these supramolecular guanosine-containing nucleopeptides and demonstrates the structural diversity of self-assembling nucleopeptides.

Keywords: Nucleopeptide • Self-assembly • Guanosine • G-quartet

Introduction

A wide range of functionalities, from acting as interactive platforms for reactions [1-3] to applications for drug delivery [4,5] have been made possible by synthetically and strategically engineered self-assembly systems made of amino acids. The fusion of short peptides with other biomolecules has been one well-documented method used by chemists and material scientists to further diversity the structures and capabilities of supramolecular systems in order to better mimic the intricate and synergistic assemblages of biological systems. One of the first systems examined to investigate these chimaera compounds was self-assembling amyloid peptides modified by nucleic acids or by lipid moieties.

The combination of nucleic acid and amino acid building blocks, known as nucleopeptides, is particularly intriguing because the amino acid component can help with supramolecular structure assembly while the nucleic acid component adds additional molecular recognition elements based on different hydrogen bonding motifs. An early example of supramolecular cooperation between peptide and nucleic acid components in a self-assembling nucleopeptide was the inclusion of the hemiprotonated C-C+ base pair, or i-motif, with an amyloid A peptide derivative to form soluble nanotubes [6]. Then, the team led by Xu created nucleopeptides that could hydrogelate on their own, both with and without additional sugar moieties. Sequence modification or the co-assembly of base-pairing nucleopeptides can change the material properties of the resultant hydrogels [7,8]. Since then, these nucleopeptides have been utilised for a variety of biomaterial applications, such as their capacity to sequester ATP in cellular settings in order to improve the effectiveness of doxorubicin treatment in cancer cells. A new library of 16 nucleopeptides (base-XFF-OH, where X is Phe, Ala, Gly, or Lys) was recently created by Suggs et al. The nucleobase and amino acid content of the nucleopeptide assemblies allowed for systematic control of the hydrogelation and subsequent mechanical characteristics, which demonstrated little cytotoxicity against 3T3 fibroblast cells.

Nucleic acids, particularly guanine, can help molecular recognition along the Hoogsteen face of the base to produce distinctive structural motifs, G-quartets and G-ribbons, in addition to Watson-Crick-Franklin hydrogen bonding. Four guanine residues are arranged cyclically in G-quartets, which hydrogen bond along the Hoogsteen face and stack to create bigger G-quadruplex structures in DNA sequences rich in G, such as telomeric sequences. Higher order G-quartet structures can also form when guanosine is inserted into self-assembling molecules, such as with lipophilic moieties and terpyridine-tethered peptide scaffolds, when coordinated by metal cations, most frequently K^+ or Na^+. The G-ribbon, a second guanosine-based motif, can combine with other guanines to form an extended sheet. It has been demonstrated that depending on the presence of metal cations, lipophilic-derivatized guanosine molecules can either form G-quartet or G-ribbon based supramolecular structures.

We created and put together a tetrapeptide (Gly-Lys-Phe-Phe) that has guanosine attached to the N-terminus, drawing inspiration from previously published nucleopeptide constructions. In this work, guanosine is referred to as gs to distinguish it from the guanine nucleobase used in earlier nucleopeptide investigations. In many short self-assembling peptide systems, the Phe-Phe-dyad is a common hydrophobic core that allows higher order assembly.

Results and Discussion

To produce a final concentration of 2 mg mL^{-1} (0.2 wt%) and a pH of 4.5, the gs-GKFF-OH nucleopeptide was constructed in 20% (v/v) acetonitrile in Millipore water. Some solutions also contained 1 equivalent of KCl to coordinate possible G-quartet structures with K^+. After 24 hours, only a little amount of the sample was still in the tube bottom during the vial inversion test, leaving behind a loose gel. But after 48 hours, a totally clear gel developed and the entire sample remained in place throughout vial inversion. For more than a week, the clear gel is stable and doesn't precipitate any nucleopeptides. The gs-GKFF-NH2 nucleopeptide was assembled using the same method. The amide C-terminal construct assembly, 2 mg mL^{-1}, continues to be in solution after 24 hours and for weeks. This nucleopeptide differs from the guanine-containing tripeptides (gua-KFF-OH and gua-GFF-OH) reported because they did not form a hydrogel and no nanofibers were characterised. However, the hydrogel formation of the guanosine-containing gs-GKFF-OH is consistent with some guanine nucleobase-containing peptides with an FF dyad.

Using Fourier transform infrared spectroscopy (FTIR) investigation of the

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amide I region (1775-1575 cm^{1}), we looked into the higher order assembly molecular interactions of the guanosine-containing nucleopeptides. There is a prominent and strong peak in the zwitterionic carboxylic acid gs-GKFF-OH nucleopeptide at 1638 cm^{1}, which is typical of sheet forming assemblies. Furthermore, the signal at 1678 cm^{1} is compatible with guanosine's C6 carbonyl participating in a hydrogen bond. A population of guanosine residues presumably participated in a weaker hydrogen-bonding environment because the modest peak at 1707 cm^{1} is nevertheless red-shifted in comparison to published peaks of the non-hydrogen bonding C6 guanosine (ca. 1724 cm^{1}). The peak at 1638 cm^{1} changes in form when KCl is added to the gs-GKFF-OH assemblies. Unique negative peak locations that resolve a shoulder at 1636 cm^{-1} in assemblies without additional KCl and a shoulder at 1645 cm^{-1} in assemblies with additional KCl are visible in the second derivative spectra of gs-GKFF-OH assemblies. The population of unordered nucleopeptide is indicated by the peak at 1645 cm^{1}. The nucleopeptide gs-GKFF-OH assemblies' peak at 1636 cm^{1} implies variability in the sheet structures.

Conclusion

The FTIR research reveals that the guanosine moieties hydrogen bond, despite the fact that the sheet forming peptide component inhibits the guanosine from forming-stacking interactions. We suggest that each G-quartet can fit into the peptide framework of the gs-GKFF-OH nucleopeptide. The prospect of nucleopeptides acting as supramolecular catalysts is made possible by the inclusion of G-quartet structures into supramolecular assemblies. Nucleopeptides containing guanosine should also be tested for emergent catalytic properties because other recent examples of guanine-based supramolecular structures have been shown to catalyse peroxidase reactions in the presence of hemin and have provided a chiral scaffold for enantiomeric Freidel-Crafts alkylation. The self-assembled guanosine-containing nucleopeptides presented in this study may also help future investigations into dynamic chemical networks because the mutualism between peptides and nucleic acids in biological systems has made them crucial systems for understanding chemical evolution.

In conclusion, two new self-assembling nucleopeptides that contain both guanosine hydrogen bonds and -sheet interactions have been created and studied. We propose that the flexibility of guanine, which can form either G-quartets or G-ribbons, works in concert with the peptide scaffold to produce different supramolecular morphologies depending on the C-terminus peptide chemistry, even though the peptide component serves as the scaffold for the higher order assembly. We are confident that the characterization of these nucleopeptides presented in this work will advance the functional applications of nucleopeptides, as further research is currently being done to elucidate additional molecular level details of these assemblies.

Acknowledgment

None.

Conflict of Interest

The author declares no conflict of interest.

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