Biophysical Chemistry 163–164 (2012) 1–10

Contents lists available at SciVerse ScienceDirect

Biophysical Chemistry

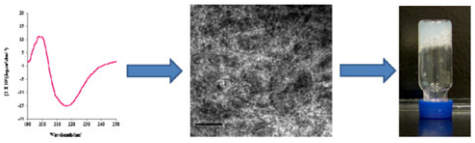
journal homepage: http://www.elsevier.com/locate/biophyschem

Structure and hydrogel formation studies on homologs of a lactoglobulin-derived peptide

Marie-Michèle Guy, Normand Voyer *

PROTEO, Quebec Research Network on Protein Structure, Function and Engineering, and Département de chimie, Université Laval, Quebec City, QC, Canada, G1V 0A6

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 13 October 2011
Received in revised form 20 December 2011
Accepted 23 December 2011
Available online 29 December 2011

Keywords:
Milk peptide
Lactoglobulin peptide
Self-assembly
β-sheet
Hydrogel formation
Secondary structure
Circular dichroism

ABSTRACT

In order to study the impact of the amino acid sequence on the morphology of peptide-based nanostructures and their hydrogel formation, we designed a series of analogs of a milk-derived octapeptide (OP), mainly using strategic amino acid substitutions. Electronic transmission microscopy (TEM) and circular dichroism (CD) spectropolarimetry were used to analyze the nanostructures formed, and to characterize some structural features of the modified peptides. Further, the potential to form hydrogels was investigated for all of the analogous peptides. We learned that those able to undergo secondary structure transition to β-sheet conformation form strong gels. The results reported highlight some key structural properties that explain the self-assembly propensity of Peptide OP.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Molecular self-assembly is ubiquitous in biological systems and consists of a spontaneous association of several individual entities into a coherent organization without external instructions. Recreating such phenomena artificially in controlled conditions has been the subject of intense research in the past decades [1–5]. The self-assembly of biomolecules such as peptides and proteins has significant implications in the biomedical sciences, materials research, and nanotechnology [6–13].

In peptide self-assembly, the initiation step is often the association of peptide units into β-sheets [14–20]. The establishment of non-covalent molecular interactions, including van der Waals, electrostatic, and hydrophobic interactions, as well as hydrogen bonding, allow the grouping and stabilization of several β-sheets together, which ultimately results in supramolecular structure formation.

Several reports have described the formation of different nanostructures, such as nanotubes, nanofibers, and hydrogels, from self-assembling peptides [9,10,13,14,16,20–25]. Peptide hydrogels are highly hydrated materials resulting from peptide nanofibers entangled together in a three-dimensional network that entraps a large amount of water molecules; this leads to gel formation [25,26]. Prospective applications of hydrogels made from β-sheet fibrillar networks include matrices for the separation of large biomolecules (such as proteins), scaffolds for cell growth and tissue engineering, encapsulation and controlled release of drugs and bioactive molecules, and templates for mineral growth [27–29]. Many factors influence the behavior of peptide self-assembly, including the amino acid sequence, concentration, pH, temperature, solvent composition, and ionic strength [30–34]. The preparation of materials via peptide self-assembly, employing both careful design of the individual molecules and selection of appropriate physico-chemical conditions, allows one to define ultimate material properties such as chemical functionality, material morphology, and mechanical and viscoelastic properties [13,20].

* Corresponding author. Tel.: +1 418 656 3613; fax: +1 418 656 7916.
E-mail address: Normand.Voyer@chm.ulaval.ca (N. Voyer).

0301-4622/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2011.12.005

Table 1
Homologs used and their modification compared to the parent OP.

Parameter	Sequence	#	Modification
Original milk peptide	L I V T Q T M K	OP	
Hydrophobic/Hydrophilic Ratio	G I V T Q T M K	H1	Decrease *N*-terminal hydrophobicity
	L L I V T Q T M K	H2	Increase *N*-terminal hydrophobicity
	L I V T Q T M A	H3	Decrease *C*-terminal hydrophilicity/Remove one + charge
	L I V T Q T K K	H4	Increase *C*-terminal hydrophilicity/Add one + charge
	L L I V T Q T K K	H5	Extend both extremities/Add one + charge
Lysine substitution (*C*-terminal extremity)	L I V T Q T M R	L1	Change side chain pKa
	L I V T Q T M E	L2	Change side chain charge
Charge distribution	Ac L I V T Q T M K	D1	Block *N*-terminal extremity/Decrease overall + charge
	L I V T Q T M K NH_2	D2	Block *C*-terminal extremity/Increase overall + charge
	K M T Q T V I L	D3	Sequence inversion/Invert charge distribution

Our group focused on the self-assembly of an octapeptide derived from the milk protein β-lactoglobulin, called Peptide OP (Table 1), which undergoes self-assembly by a secondary structure transition to β-sheet conformation in a solution at basic pH [35]. Peptide OP with an amino acid sequence LIVTQTMK is amphiphilic, with a hydrophobic part located at the N-terminal extremity and a hydrophilic lysine residue at the C-terminal extremity. Previous work revealed that Peptide OP self-assembly and hydrogel formation can be triggered significantly by pH and peptide concentration variation, whereas temperature and ionic strength variations have little influence on these processes [36]. On that basis, we proposed that the self-assembly of Peptide OP is driven by β-sheet formation resulting from hydrophobic packing, establishment of stabilizing hydrogen bonds, and the right balance of attractive and repulsive electrostatic interactions.

Despite our increased understanding of the peptide self-assembly mechanism gathered from different studies [13,20,23], there still remains much to learn about the design and self-assembly of peptides, more specifically about the relationship between the peptide's primary structure and the resulting morphology of the self-assembled nanostructures [32,37,38]. Taking into account the potential applications of peptide hydrogels originating from an edible/biocompatible source, the aim of this work is to gain insight about the structure-assembly relationship of Peptide OP by studying the impact of the amino acid sequence on the nanostructure's conformation, morphology, and hydrogel formation.

In order to achieve this, we designed a series of OP homologs by using strategic amino acid substitutions (Table 1). Since the amphiphilic character of self-assembling peptides appeared to be a key structural determinant to drive self-assembly, we first synthesized a group of peptides with different ratios of hydrophobic and hydrophilic residues (Table 1, H1–H5). Also, as several studies reported on the implication of electrostatic repulsive and attractive interactions arising from lysine residues in peptide self-assembly [8,15,16,33,38,39], we also prepared homologs that had only the lysine residue substituted by other charged amino acids (Table 1, L1 and L2).

Then, to further study the implication of electrostatic repulsive and attractive interactions, we synthesized a group of peptides with identical amino acids to the milk-derived OP, but with different charge distributions (Table 1, D1–D3). Many physico-chemical conditions trigger and control peptide self-assembly, but we chose to focus on pH in this study, since our previous works showed that the self-assembly of octapeptide is strongly dependent on pH variations [35,36].

Circular dichroism spectropolarimetry (CD) was used to study the secondary structure of the modified peptides at different pH values in order to determine which amino acid substitutions induce or inhibit β-sheet formation and therefore self-assembly. Transmission electron microscopy (TEM) was used to observe the impact of amino acid modifications on nanostructure morphology. Further, the potential to form hydrogels was investigated for all the homologous peptides able to undergo secondary structure transition to β-sheet conformation. Hydrogel formation was triggered by pH variations and hydrogels were then characterized using visual observations. The overall outcome of this work is to move toward a better understand of the unique gelation properties of naturally derived peptides and to refine even further the molecular detail of the self-assembly process in order to design better materials especially suitable for industrial applications.

2. Materials and methods

2.1. Materials

All peptides were prepared using standard solid-phase Fmoc strategy and synthesized on Wang resin, except for Peptide D2 that was synthesized on Rink resin. The 2-(1H-benzotriazol1-yl)-1,1,3,3-tetramethyl uranium hexafluorophosphate/1-hydroxy-benzotriazole (HBTU/HOBT) mixture was used as the coupling reagent. The amine group deprotection cycles were achieved using a solution of 20% piperidine in N,N-dimethylformamide(DMF). The resulting resin-bound peptides were cleaved and side-chain deprotected by use of trifluoroacetic acid (TFA)/water (H_2O)/1,2-ethane dithiol (EDT)/triisopropylsilane (TIS) (94:2.5:2.5:1). In order to recover the peptides and eliminate impurities, the peptides were triturated 5 times with petroleum ether and then vacuum-dried. All peptides were characterized by HPLC and ESI mass spectrometry.

2.2. Circular dichroism (CD) spectropolarimetry

Analyses were performed using a Jasco J-710 instrument (upgraded to a J-715) with 1.08.01 spectral management software using freshly prepared aqueous solutions of peptides with a concentration of 2 mg/mL. The solubilization of peptides was improved by combining the addition of 10% (v/v) of 2,2,2-trifluoroethanol (TFE) with a 20 minute ultrasonic treatment. Spectra were recorded at 22 °C in quartz cylindrical cells with a 0.01 cm path length. Ten scans were collected from 250 to 190 nm with a data pitch of 0.2 nm and a scanning speed of 100 nm/min. For all CD experiments, the pH adjustments were done using NaOH and HCl solutions. Amounts added for pH adjustments were taken into account in the calculation of the final peptide concentration. All CD measurements were duplicated. The resulting data were background-corrected and smoothed.

2.3. Transmission electron microscopy

Samples were prepared by rehydrating lyophilized peptides to a concentration of 5 mM and agitating for 1 h. The pH was then adjusted to 10.0 and samples were left to rest for 30 min to allow nanostructure formation at higher peptide concentrations. The peptide solutions were then diluted to a final peptide concentration of 0.50 mM and the pH was readjusted for each solution. 2 μL of each sample was applied to a carbon-coated copper grid and air-dried. The samples were negatively stained with 2 μL of 1% uranyl acetate solution and air-dried. Specimens were examined with a JEM 1230

Download English Version:

https://daneshyari.com/en/article/5371212

Download Persian Version:

https://daneshyari.com/article/5371212

Daneshyari.com