ELSEVIER

Nanomedicine: Nanotechnology, Biology, and Medicine
xx (2018) xxx–xxx

nanomedicine
Nanotechnology, Biology, and Medicine

nanomedjournal.com

Large supramolecular structures of 33-mer gliadin peptide activate toll-like receptors in macrophages

María Georgina Herrera, PhD[a,1], Malvina Pizzuto, PhD[b,1], Caroline Lonez, PhD[b,1], Karsten Rott, PhD[c], Andreas Hütten[c], Norbert Sewald[a], Jean-Marie Ruysschaert, PhD[b], Veronica Isabel Dodero, PhD[a,*]

[a]*Universität Bielefeld, Fakultät für Chemie, Organische Chemie, Universitätsstr. 25, 33615, Bielefeld, Germany*
[b]*Structure and Function of Biological Membranes, Université Libre de Bruxelles, Belgium*
[c]*Universität Bielefeld, Fakultät für Physik, Universitätsstr. 25, 33615, Bielefeld, Germany*

Received 12 October 2017; accepted 16 April 2018

Abstract

Gliadin, an immunogenic protein present in wheat, is not fully degraded by humans and after the normal gastric and pancreatic digestion, the immunodominant 33-mer gliadin peptide remains unprocessed. The 33-mer gliadin peptide is found in human faeces and urine, proving not only its proteolytic resistance *in vivo* but more importantly its transport through the entire human body. Here, we demonstrate that 33-mer supramolecular structures larger than 220 nm induce the overexpression of nuclear factor kappa B (NF-κB) via a specific Toll-like Receptor (TLR) 2 and (TLR) 4 dependent pathway and the secretion of pro-inflammatory cytokines such as IP-10/CXCL10 and TNF-α. Using helium ion microscopy, we elucidated the initial stages of oligomerisation of 33-mer gliadin peptide, showing that rod-like oligomers are nucleation sites for protofilament formation. The relevance of the 33-mer supramolecular structures in the early stages of the disease is paving new perspectives in the understanding of gluten-related disorders.

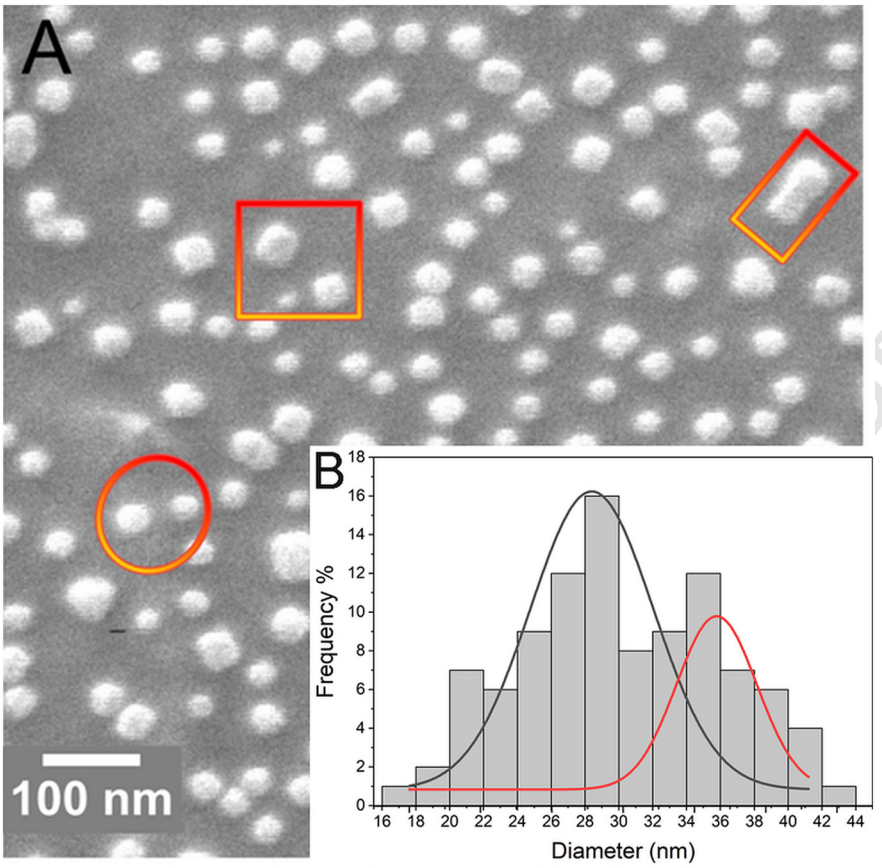
Key words: Oligomers; Gluten-related disorders; Celiac disease; Innate immune response; Helium ion microscopy

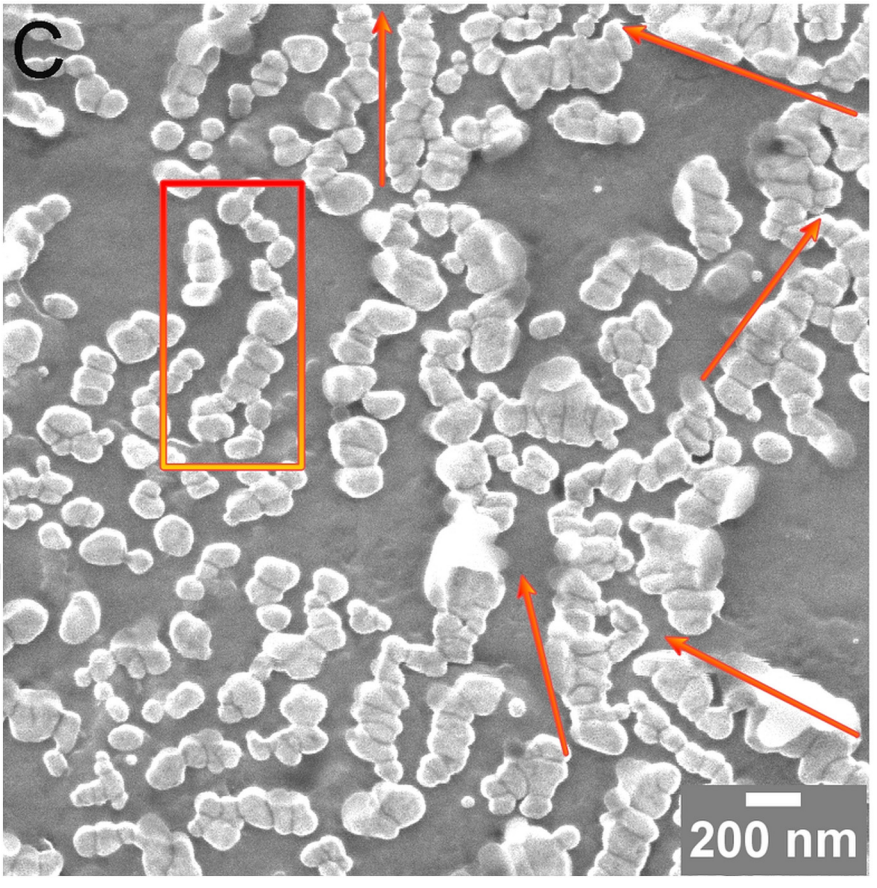
Gluten is a complex protein matrix present in wheat, barley, rye and some varieties of oats that elicits complex immunological disorders, which affect around 1 to 7% of the global population. They are named according to clinical symptoms and immunological response as wheat allergy, celiac disease (CD), gluten ataxia, dermatitis herpetiformis and non-celiac gluten sensibility.[1] In wheat, gliadin and glutenin are the major components of gluten.[2] It is known that humans do not fully degrade gliadin and after its digestion, some large peptic fragments remain unprocessed. One of these fragments is the immunodominant 33-mer gliadin peptide.[3] This protein fragment has also been found in human faeces and urine, proving not only its proteolytic resistance *in vivo* but more importantly its transport through the entire human system.[4] From a molecular point of view, this peptide behaves as a non-ionic amphiphile which oligomerizes into soluble nanostructures of different sizes which coexist in equilibrium as determined by electron microscopy (EM), atomic force microscopy (AFM), and dynamic light scattering (DLS).[5,6] Recently, a radiolabelled mutated ^{3}H-33-mer and its oligomers were found in blood plasma and accumulated in different organs in murine models after oral administration.[7] Moreover, the 33-mer is present in various wheat flours at levels ranging from 91 to 603 μg/g flour.[8]

Up to now, the 33-mer peptide activity has been linked mainly to the adaptive immune response[9] but the activation of the innate response remains poorly understood.[10] Analysis of intestinal mucosa from untreated celiac patients[11] and various

Competing financial interests: The authors declare no competing financial interests.
Final Support: Alexander von Humboldt Foundation (HERMES), DAAD (German Academic Exchange Service), FRIA (FNRS) and foundation Wiener-Anspach and the Marie Curie Actions (TLR4-CAT PIEF-GA-2012-326481).
*Corresponding author.
E-mail address: veronica.dodero@uni-bielefeld.de. (V.I. Dodero).
[1] Those authors contributed equally to the work.

https://doi.org/10.1016/j.nano.2018.04.014
1549-9634/


A
100 nm
B
Frequency %
Diameter (nm)
0
2
4
6
8
10
12
14
16
18
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44


C
200 nm