Review

Formation mechanisms, handling and digestibility of food protein nanofibrils

Saina Moayezzadeh\textsuperscript{a}, Ashkan Madadlou\textsuperscript{b,c,d,*} and Asghar Khosrowshahi asl\textsuperscript{a}

\textsuperscript{a}Department of Food Science and Engineering, College of Agriculture, Urmia University, Urmia, Iran
\textsuperscript{b}Department of Food Science and Engineering, University College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran
\textsuperscript{c}Center of Excellence for Application of Modern Technologies for Producing Functional Foods and Drinks (FFDCE), University College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran
\textsuperscript{d}Interdisciplinary Research Department of Agricultural and Natural Resources Nanotechnology (IRDANN), University College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

\textsuperscript{*} Corresponding author.

Background: Globular proteins including whey proteins, soy proteins and egg white proteins self-assemble into fibrillar structures with several nanometers thickness and several micrometers length by prolonged heating at very acidic conditions. These \textit{in vitro} synthesized fibrils resemble amyloids associated with various neurodegenerative diseases and hence have received special attention by bioscientists. The synthesized fibrils are however of widespread potential for food and colloid sciences applications. Characteristics such as aspect ratio, and persistence and contour lengths of the artificially-synthesized fibrils could be elaborated by appropriate selection and employment of parameters of fibrils formation process.

Scope and approach: The fibrillation process of whey proteins, proposed mechanisms and potent inhibitors of the process, as well as, alternative enzyme-based routes of fibrillation are overviewed in the present article. Then post-formation treatment and applications of globular proteins fibrils and their gastric digestibility behavior are briefly referred followed by representing some future trends in this field.

Key findings and conclusions: The building units of protein fibrils are hydrolysis-generated polypeptides rather than parent intact protein monomers. It was hypothesized that proteins fibrillation is an oxidation-triggered process and may be inhibited by antioxidant agents that suppress the generation of reactive oxygen species. Whey protein-originated fibrils may be exploited in formation of heat-resistant protein-stabilized emulsions and nanoemulsions. However, more effort is required to characterize the interfacial behavior of fibrils in comparison with native and heat-denatured whey proteins. Gastric and intestinal digestion fate of protein nanofibrils is also a hot topic for upcoming research studies.

Keywords: Protein nanofibril; Whey proteins; Amyloid; Mechanism; Reactive oxygen species; Digestibility

Introduction

Heat denaturation of proteins can form a wide variety of structures such as fibrils, flexible strands, branched structures and random aggregate (Bolder, Hendrickx, Sagis, & van der Linden, 2006) depending on the pH value, salt type and concentration, heating conditions and protein concentration (Nicolai, Britten, & Schmitt, 2011), among which, the greatest impact belongs to heat (Pearce, Mackintosh, & Gerrard, 2007). The pH value of protein solution is also of profound influence on the organizational arrangement of super-structures. The architecture and life span of these structures are determined by balance between attractive hydrophobic and repulsive electrostatic interactions. At low ionic strengths and at pH values far from the iso-electric point (pI), electrostatic repulsive forces overcome the attractive interactions resulting in formation...
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diameter); whilst, at pH values close to pI, spherical micro-
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In the latter scope, fibrillation is deliberately induced for
the molecular mass of 18400 Da and radius of 7.5%
of fibrillar structures with high aspect ratio (length versus
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tures has received considerable attention owing to the abil-
ity of the fabricated structures to display novel profitable
characteristics (van der Linden, 2006). In the assembly pro-
cess, protein monomers are organized into giant supramole-
cules in which non-covalent interactions such as hydrogen
bonds, and electrostatic, hydrophobic and van der Waals in-
teractions hold the building units together (Bolder, 2007).
Fibrils formation may involve covalent linkages (disulfide
bridges) in addition to non-covalent interactions depending
on pH. Heat treatment at pH 3.35 assembled fibrils not only
by hydrogen bonds and hydrophobic interactions but also
by limited number of disulfide bridges; whilst, by heating at
pH 2.0 merely non-covalent hydrophobic interactions
contributed in fibrils formation (Mudgal, Daubert, Clare,
& Foegeding, 2010).

The essential characteristics of fibrils
Several types of food proteins such as milk proteins, soy
proteins and egg white proteins can assemble in vitro at
appropriate condition into fibrillar structures with micro-
-meter length and nanometer thickness (Bolder, 2007; van
der Linden, 2006). These objects resemble amyloid fibrils
that are associated with neurodegenerative diseases like
Alzheimer’s, Parkinson’s, Huntington’s and Creutz-
fel dt—Jakob diseases. Accordingly they are often called
“amyloid-like” (Jones & Mezzenga, 2012). The amyloid-
like nanofibrils are of scientific interest to investigate their
role and/or application in diverse areas such as biomedicine
that the inhibition of nanofibrillation is desired, as well as
in molecular biology, material science, and food science.
In the latter scope, fibrillation is deliberately induced for
obtaining purposive functionalities (Zhao, Pan, & Lu,
2008).

The properties of the fibrils depend on length distribu-
tion and their aspect ratio. For example, a high aspect ratio
may lead to an entangled network at much lower protein
concentrations (van der Linden & Venema, 2013). These
features propose a variety of applications for protein nano-
fibrils in food industry including utilization as efficient
thickeners, gelators, foaming agents, stabilizers of foams
and emulsions, and enzyme immobilization matrices, as
well as, for encapsulation purposes and fabrication of bio-
sensors (Nicolai et al., 2011). It is important to control and
elaborate the length distribution of nanofibrils. Two length
scales are used to characterize fibrils morphology: persis-
tence length (Lp) and contour length (Lc). The former ex-
hibits fibril flexibility, while the latter is fibril’s length at
maximum extension. When Lp << Lc, fibril is flexible; whilst,
when Lp >> Lc, fibril is rigid. When Lp and Lc have comparable magnitudes fibrils are regarded semi-
flexible (Loveday, Rao, Creamer, & Singh, 2009). Mudgal,
Daubert, and Foegeding (2009) randomly selected four to five β-lactoglobulin fibrils from different quadrants of
transmission electron microscopy (TEM) images and
digitized the fibrils along their contours into small seg-
ments. Summation of the length of all segments was
considered as Lc and the bond angles correlation along
the contour was employed for Lp calculation. They found
that heating of β-lactoglobulin solution at pH 3.35 yielded
more flexible fibrils compared to pH 2.0 which was evi-
denced with shortened persistence length from 788 nm (at
pH 2.0) to 35 nm (at pH 3.35).

vandenAkker, Engel, Velikov, Bonn, and Koenderink
(2011) intended to link the mechanical properties of fibrils
to their molecular structures, in order to gain appropriate
understanding of fibrils assembly process. At low protein
concentration, straight fibrils with high persistence length
and well-developed β-sheet structures formed while at
high protein concentration flexible fibrils with shorter
persistence length and high α-helix content generated.
The persistence length of fibrils varied from about
3818 nm at 3% β-lactoglobulin concentration to 92 nm at
7.5% β-lactoglobulin concentration. The much longer
persistence length of straight fibrils originated from a
network by hydrogen linkages in the linear backbone of
β-sheet-rich fibrils. Accurate measurement of length distrib-
ution of nanofibrils is crucial for fundamental understand-
ing and controlling of the fibrillation process in order to
develop efficient therapies against amyloid-contributed dis-
eases. It would also make possible to determine the me-
chanical characteristics and biological impacts of
amyloid-like fibrils generated artificially as potential
nano-materials. The Weibull distribution model applied
for describing the length of human β2-microglobulin linear
fibrils that had been imaged by a tapping-mode atomic
force microscope revealed that fibril fragmentation, a
mechanism that shortens fibrils is of significant influence
on prions replication and phenotype (Xue, Homans, &
Radford, 2009).

It is essential to have an accurate understanding of the
formation process and properties of the fibrils and to have
ability to control these properties and consequently the
functionality of the fibrils for successful application in
various products. Herein, the assembly behavior of globular
food proteins specially β-lactoglobulin and the properties
of the generated fibrils are comprehensively reviewed.

Fibrillation process
Process overview
Beta-lactoglobulin is a globular whey protein with mo-
lecular mass of 18400 Da and radius of ~2 nm (Bolder,
Vasbinder, Sagis, & van der Linden, 2007). It exists in three
genetic variants A, B and C which differ from each other at
the substitutions in their amino acid sequences. The vari-
ants A and B contain Gln at residue 59 while variant C con-
tains His. As well, variant A contains respectively Asp and