Advances in Colloid and Interface Science 239 (2017) 115–126

Contents lists available at ScienceDirect

Advances in Colloid and Interface Science

journal homepage: www.elsevier.com/locate/cis

Heteroprotein complex coacervation: A generic process

Thomas Croguennec [a], Guilherme M. Tavares [a,b], Saïd Bouhallab [a,*]

[a] STLO, UMR1253, INRA, Agrocampus Ouest, 65 rue de Saint Brieuc, F-35042 Rennes, France
[b] Laboratory of Research in Milk Products, Universidade Federal de Viçosa, BR-36570 Viçosa, Brazil

ARTICLE INFO

Available online 17 June 2016

Keywords:
Complex coacervation
Heteroproteins
Globular proteins
Unstructured proteins

ABSTRACT

Proteins exhibit a rich diversity of functional, physico-chemical and biodegradable properties which makes them appealing for various applications in the food and non-food sectors. Such properties are attributed to their ability to interact and assemble into a diversity of supramolecular structures. The present review addresses the updated research progress in the recent field of complex coacervation made from mixtures of oppositely charged proteins (i.e. heteroprotein systems). First, we describe briefly the main proteins used for heteroprotein coacervation. Then, through some selected examples, we illustrate the particularity and specificity of each heteroprotein system and the requirements that drive optimal assembly into coacervates. Finally, possible and promising applications of heteroprotein coacervates are mentioned.

Contents

* Corresponding author. Tel.: +33 2 23 48 57 42; fax +33 2 23 48 53 50.
E-mail address: said.bouhallab@rennes.inra.fr (S. Bouhallab).

http://dx.doi.org/10.1016/j.cis.2016.06.009
0001-8686/

1. Introduction

Proteins are natural polymers (biopolymers) that share properties common to amphiphilic molecules and polyampholytes due to the chemical properties of their elementary building units. This enables proteins to interact with molecules having different properties (i.e. hydrophobic, hydrophilic and/or charged molecules, etc.) but is also at the source of specific interaction properties due to specific surface anisotropies at a molecular level. Consequently, proteins are the scaffolding of a large diversity of nano- and microstructures formed throughout diverse interactions between proteins or proteins and other components depending on the physico-chemical conditions. Amorphous reversible or irreversible aggregates, fibers, ribbons, hydrogels and complex coacervates are examples of formed nano- and microstructures [1–5]. The formation of complex coacervates consequently to protein–polysaccharide interactions is a well-documented process since the pioneering studies on gelatin and a polysaccharide by Bungenberg de Jong and Kruyt [6–8]. Complex coacervation is a liquid–liquid phase separation driven by electrostatic interactions between two oppositely charged macromolecules and by the entropic gain linked to the release of macromolecule-associated counter-ions. After complete phase separation, a lower concentrated phase (coacervates) is formed in equilibrium with an upper dilute (lean) phase [9,10]. Numerous studies deal with complex coacervation involving proteins and synthetic polyelectrolytes or polysaccharides but much less works are reported on systems containing proteins especially globular proteins [11]. The present review focuses on the complex coacervation in heteroprotein systems, trying to highlight their specificity and originality.

Heteroprotein complex coacervates made of oppositely charged globular proteins correspond to a specific balance of short-range and long-range coulombic interactions between proteins. Otherwise, proteins either form an amorphous precipitate or remain in solution. As such, coacervates are observed in very narrow ranges of pH, ionic strength, protein concentration and protein stoichiometry. The heteroprotein complex coacervation with globular proteins usually forms at low ionic strength and in a pH range lower than unity in between the pI of the two proteins, even if due to protein charge anisotropy complexation could theoretically form when the proteins carry the same net charge. Another important requirement is protein stoichiometry in order to respect the charge and size compensation principle [12]. The extreme sensitivity to change in pH and ionic strength suggests the predominant role of the electrostatic interactions in the formation of the coacervate even if dipolar attraction and hydrophobic interactions are also cited as driving forces in heteroprotein coacervation [13]. In the presence of salt the coulombic interactions between the proteins and the entropic contribution due to counter-ions release are reduced. However, the entropic forces related to counter-ions release associated with complex coacervation are supposed to be significantly lower for heteroprotein systems than for polyelectrolyte systems because of the lower charge density for the former. Heteroprotein complex coacervation is fully reversible when the balance of attractive and repulsive forces deviates from optimum conditions that constitute an essential property with delivery system perspective in mind. Constrained structural feature and surface charge anisotropy (surface charge density, size and shape of the patches) orient the interactions to specific domains on the surface of the proteins [14,15]. This constitutes specificities compared to complex coacervation involving polyelectrolytes and explain for a part that the (+/−) stoichiometry is not a sufficient condition for heteroprotein coacervation. Anisotropic interactions may determine coacervate stoichiometry (and stoichiometry of the primary complexes of the coacervate) and consequently explain the limited coacervation conditions and its extreme sensitivity to variation in the physico-chemical conditions.

The field of heteroprotein coacervation is developing rapidly during the last ten years thanks to versatile properties of the proteins. The studies aimed to establish the main coacervation mechanisms using a diversity of protein binary systems and with incorporation of sophisticated biophysical techniques. This report aims at reviewing the current state of the art in the field of heteroprotein coacervation involving one basic, positively charged protein and one acidic, negatively charged protein. The main scientific elements are described in two parts based on the two major basic proteins studied, lactoferrin and lysozyme. After a brief description of the properties of the used proteins, we describe the lactoferrin based coacervates, then summarize the work on lysozyme based coacervates, the third part being devoted to a summary of the studies conducted with other binary systems.

2. “Identity card” of studied proteins

Several basic and acidic proteins were used to study the complex coacervation in different heteroprotein systems. Table 1 summarizes the properties of the main used proteins.

2.1. Avidin

Avidin (AVI) is found in egg white of reptiles, amphibians, and birds. Chicken avidin is known to be an indispensable protein for extensive types of biotechnological application [16]. AVI molecule is a basic non covalent homotetrameric glycoprotein with a pI of 9.5. The monomers contain 128 amino acid residues, giving the protein an overall molecular weight of about 57 kDa. 10% of the overall molecular weight is attributed to carbohydrate moieties. Although each monomer shares interactions with the three others, AVI is usually regarded as a dimer of dimers. The dimers are constituted of monomers highly stabilized by a multitude of polar and hydrophobic interactions, and the interaction

Table 1
Physicochemical properties of the reviewed globular [A] or unstructured [B] proteins and polypeptides.

Structure	Protein	Abbreviation in the text	Origin	Isoelectric point	Molecular weight (kDa)
A	Lactoferrin	LF	Milk	8.7	83
A	Lysozyme	LYS	Egg white	10.7	14.3
A	Avidin	AVI	Egg white	9.5	4 × 14.3
A	Napin	NAP	Rapseed	>10	14
B	Gelatin A	GelA	Animals	9	Highly variable
B	Polylysine	PLys	Synthesis	>9.5	On demand
B	α-Casein	α-CN	Milk	5.0	24
B	β-Casein	β-CN	Milk	5.4	24
B	κ-Casein	κ-CN	Milk	5.6	19
A	α-Lactalbumin	α-LA	Milk	4.3–4.7	14.2
A	β-Lactoglobulin	β-LG	Milk	5.2	2 × 18.3
A	Bovine serum albumin	BSA	Milk	5.0	66.3
A	Osteopontin	OST	Milk	3.6	36
A	Ovalbumin	OVA	Egg white	4.5	45.5
B	Gelatin B	GelB	Animals	4.5–5	Highly variable
B	Polyglutamic	PGlu	Synthesis	<4	On demand