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Historical perspective

Bulk and nanoscale polypeptide based polyelectrolyte complexes

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ABSTRACT

Polyelectrolyte complexes (PECs) formed using polypeptides have great potential for developing new self-assembled materials, in particular for the development of drug and gene delivery vehicles. This review discusses the latest advancements in PECs formed using polypeptides as the polyanion and/or the polycation in both polyelectrolyte complexes that form bulk materials and block copolymer complexes that form nanoscale assemblies such as PEC micelles and other self-assembled structures. We highlight the importance of secondary structure formation between homogeneous polypeptide complexes, which, unlike PECs formed using other polymers, introduces additional intermolecular interactions in the form of hydrogen bonding, which may influence precipitation over coacervation. However, we still include heterogeneous complexes consisting of polypeptides and other polymers such as nucleic acids, sugars, and other synthetic polyelectrolytes. Special attention is given to complexes formed using nucleic acids as polyanions and polypeptides as polycations and their potential for delivery applications.

Contents

1. Introduction

Polyelectrolyte complexes (PECs) are formed by mixing oppositely charged polyelectrolytes, which can occur by creating a composite material that macrophase or microphase separates from solution [1,2], adsorbs sequentially on a surface as is the case in electrostatic layer-by-layer assembly [3,4], or on the surface of "hairy colloids" containing grafted polyelectrolytes [5], the former of which is the main focus of this review. Although related phenomena, this definition does not include the mixing of polymers with oppositely charged colloids [6,7] or surfactants [8] since both components are not polymeric. Polyelectrolyte

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complexation is entropically driven by the release of counterions initially confined to the double layer, which occurs when the oppositely charged polymers come together [9,10]. The nature of these phase separated complexes can be solid, in the form of a precipitate, or liquid, in the form of a coacervate [1]. Precipitates have found limited utility as functional materials without special processing, such as the addition of salt [11]. In contrast, the coacervate phase contains very high water content [12] and has very low interfacial tension with water. [13–15] These properties make coacervates important in a variety of different applications and scenarios. For example, coacervates are used to encapsulate flavors and additives in the food industry [16], as underwater adhesives for marine organisms [17], have been implicated in origin of life scenarios [18–20], used as electronic ink [21], in direct ink writing [22,23], and as potential drug delivery vehicles [2,24]. Polyelectrolyte complexation can occur as either a macroscopic phase separation that forms bulk materials or a microphase separation when block copolymers are used, enabling the formation of nanoscale PECs [2,9,25].

This review focuses on PECs formed as bulk and nanoscale materials using polypeptide based polyelectrolytes. Numerous studies have focused on using complex biopolymers and proteins to study PEC formation, which have the benefit of being biocompatible, but are structurally complex [26]. Polypeptides provide an interesting alternative to these complex biopolymers, due to their inherent biocompatibility (excluding high concentrations and molecular weights of polycationic polypeptides [27]) and simple polymeric structure, which can allow for a more systematic investigation into PEC phenomena. Moreover, polypeptides can form secondary structure motifs that can mimic protein behavior and introduce additional intermolecular forces such as hydrogen bonding that influence the stability and phase behavior of PECs [28,29].

Polypeptide based materials can be synthesized using standard fluorenylmethyloxycarbonyl chloride (FMOC) based solid phase synthesis [30,31]. This technique grows the polypeptide chain on a porous polystyrene support one amino acid at a time, allowing for the polypeptides to have specific charge distributions and sequences that are not accessible using other polymerization methods [30,31]. In addition, the polypeptides prepared using solid phase synthesis have relatively low polydispersities compared to other polymerization methods, but relatively low molecular weights of less than 100 amino acids. To create block copolymer polypeptides, polymers containing carboxylic acid functional groups can be conjugated to the polypeptide chains [32]. For higher molecular weight polypeptides, *N*-carboxyanhydride (NCA) ring opening polymerization is used, which can create polypeptide chains larger than 200 amino acids in length. This technique facilitates large scale synthesis of polypeptides in gram quantities and has benefited from recent advancements in novel catalysts and better NCA purification [33]. Block copolymers containing polypeptide segments can be synthesized using this method by initiating the polymerization from a polymer containing a terminal amine functionality [33].

2. Bulk polyelectrolyte complexes

Mixing oppositely charged polyelectrolytes results in the formation of bulk PECs. Liquid complexes, or coacervates are characterized by the formation of micron sized spherical droplets that can coalesce and form a distinct phase [34]. On the other hand, precipitates form irregularly shaped amorphous solids that can be re-suspended after centrifugation [12]. Images of both types of complexes can be observed in Fig. 1. This section discusses the formation of bulk PECs formed using a polypeptide as the polycation, polyanion, or both.

2.1. Homogeneous polypeptide complexes

Homogeneous PECs are formed when both the polyanion and polycation are polypeptides. An extensive study on PECs using polylysine (pLys) and polyglutamic acid (pGlu) investigated the effects of pH, temperature, molecular weight, polyanion/polycation mixing ratio and total polymer concentration on the PEC formation phase diagram, concluding that polypeptide based complexes have similar tendencies to other PEC systems [12]. A related study probed the thermodynamic driving forces of polypeptide complexation using isothermal titration calorimetry and found that the process was entropically driven and endothermic [10]. The interfacial energy of homogeneous polypeptide coacervates has been measured to be extremely low (<1 mN/m) using a surface forces apparatus [14], which is also comparable to other coacervate systems [13].

Choosing polypeptides as polyions allows for very precise control over charge spacing and polydispersity, especially when synthesized using solid phase methods. An advanced Monte Carlo simulation with a replica exchange algorithm combined with experimental techniques allowed for the investigation of charge distribution and charge matching in three model systems that use glycine residues as spacers in both lysine and glutamic acid based polyelectrolytes: $(EGEG)_5$/$(KGKG)_5$, $(EEGG)_5$/$(KKGG)_5$, and $(EEGG)_5$/$(KGKG)_5$ [35]. Zhao and colleagues conclude that the size, density, and packing of the complexes can be tuned with the distribution of neutral spacers, such that the $(EGEG)_5$/$(KGKG)_5$ complexes, where the charged residues alternate 1-to-1 with the neutral residues, have the highest density and molecular weight found using laser light scattering, and this is in agreement with the largest aggregation number found using the simulations [35]. Interestingly, the density, molecular weight, and aggregation number of the mismatched complex, $(EEGG)_5$/$(KGKG)_5$, was higher than both of the matched cases, indicating that charge matching also influences the complexation phenomena [35].

Polypeptide based polyelectrolytes can be synthesized in gram scale quantities using NCA ring opening polymerization methods [33]. These methods do not allow for precise control over the sequence, but can still form molecules of interest, including incorporating amino acids in the same proportions as those found in sand castle worm proteins, which form coacervates with exceptional adhesive properties [17]. Zhang et al. created a cationic polypeptide consisting of lysine, dihydroxyphenylalanine (DOPA), glycine, and tyrosine and an anionic polypeptide consisting of phosphoserine, serine, and tyrosine; both in gram scale quantities. Mixing these polypeptides together created liquid coacervate complexes with low surface tension, shear-thinning behavior and a viscoelastic response, providing a robust synthetic method for creating biomimetic materials [36].

One of the unique properties of polypeptide based complexes involves the ability for secondary structure formation in the form of β-sheets and α-helices. Recently, it has been shown that homochiral polypeptide based complexes form β-sheet structures that facilitate the formation of solid complexes, while racemic polypeptides inhibit β-sheet formation due to steric hindrance and create liquid complexes, illustrating mechanisms to alter PEC phase behavior using secondary structure formation [28,37]. This work included both experimental results and molecular dynamics simulations that illustrated the same conformations [28,37]. Images of the complexes formed can be seen in Fig. 1. Using synthetic techniques that were designed to increase the spacing between the charged group and the polypeptide backbone allowed the creation of polypeptides that were simultaneously charged and α-helical [38]. These helical polyelectrolytes formed liquid coacervate phases when mixed with polyglutamic acid (pGlu), regardless of the polyanion chirality due to the reduced intermolecular hydrogen bonding arising from the intramolecular α-helical structure [29]. Interestingly, the critical salt concentration of the coacervates containing helical polypeptides is extremely high compared to regular ionic polypeptide complexes, likely due to the increased charge density of the helical polyelectrolyte [29].

2.2. Heterogeneous polypeptide complexes

Heterogeneous polypeptide complexes may be formed by mixing a polypeptide with synthetic polymers or biopolymers. However, most

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