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Extracellular aggregation of polyelectrolytes escaped from the cell interior: Mechanisms and physiological consequences

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ABSTRACT

The cell interior is rich in anionic polyelectrolyte filaments. They are normally prevented from aggregating non-specifically with polyvalent counterions by multiple mechanisms involving specific protein ligands and dynamic processes far from equilibrium. In contrast, extracellular protein filaments are generally not polyelectrolytes, and extracellular fluids are rich in polycations but devoid of ATP-consuming dynamic regulation of polymer assembly. As a result, when intracellular polyelectrolytes like DNA or F-actin enter the extracellular space they often form large bundles with polycationic peptides or other factors, some of which are required for defense against bacteria. Formation of polyelectrolyte aggregates can have not only some beneficial effects, but also many harmful effects related to changing extracellular fluid viscoelasticity and antimicrobial function.

1. Introduction

Biopolymers with high charge density are common in biology. The polyelectrolyte properties of rigid and semi-flexible filamentous biopolymers have large effects on their self-assembly in vitro, and very likely help mediate their assembly in vivo. The best-studied biological polyelectrolyte is double-stranded DNA [1•,2], but also, of the six most abundant protein filaments in the body, three are strong polyelectrolytes with surface charge densities at least as great as those of DNA. F-actin, microtubules, and intermediate filaments, which form the intracellular cytoskeleton are strong anionic polyelectrolytes. In contrast to the high surface charge of intracellular protein filaments, none of the three most abundant protein filaments in the extracellular space, collagen, fibrin, and elastin are polyelectrolytes [3]. There are extracellular polyelectrolytes, loosely defined as linear polymers with spacing between fixed charges (almost always negative) less than the Bjerrum length (Fig. 1), but these are all flexible polysaccharides or brush-like proteoglycans in which the fixed charges are carried by flexible polymer backbones [4••]. Some anionic proteins are too small to function as linear polyelectrolytes themselves, but as in the case of hyperphosphorylated tau [5] or α-synuclein [6], they can form amyloid fibers or other forms of linear polymer, which then interact with oppositely charged ligands and surfaces [5,7]. Large condensed bundles of polyelectrolytes that form in the presence of multivalent counterions are generally not observed in cells, even though the concentrations of polyelectrolytes and the concentrations of polyvalent counterions are probably above the threshold needed for this condensation to occur, largely because the intracellular system is generally kept far from equilibrium by energy-consuming metabolic reactions [8].

When intracellular polyelectrolytes escape from the cell during trauma or necrosis and enter the extracellular fluids, their interactions with soluble counterions in the extracellular space produce a wide variety of large aggregates. They assemble not by specific cross-linking ligands, but largely because of polyelectrolyte effects, especially the release of small valence counterions as larger valence polycations in the intracellular space dock to their newly added polyelectrolyte targets. Some of the aggregates formed between extracellular polycations and intracellularly-derived anionic polyelectrolytes form complexes that have specific and advantageous signaling effects, especially on the immune system [9•], but many interactions of intracellular polyelectrolytes with extracellular ligands cause pathologies due to the assembly of these very large polyelectrolyte complexes [5,6,10–12] or to inactivation of the physiological functions of cationic molecules that become entrapped within them.

Polyelectrolyte aggregates are formed by a number of polycations including metal ions, polyamines and cationic antibacterial peptides (CAPs) and are implicated in diseases such as cystic fibrosis (CF), lung infection, abscess formation, autoimmune and neurodegenerative disorders and cancers. This review highlights recent advances in

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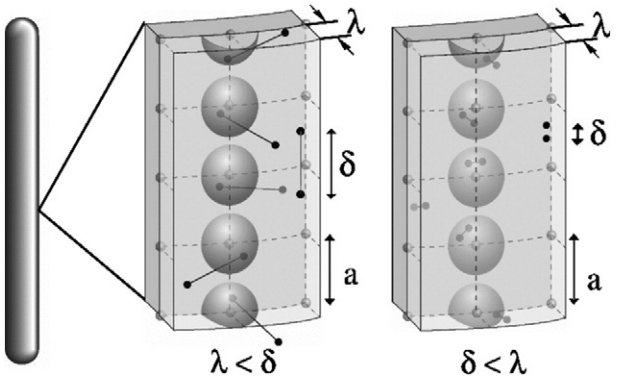

Fig. 1. Requirement for divalent counterions to aggregate polyelectrolyte cylinders. The polyelectrolyte filament is characterized by a spacing between fixed unitary charges, usually negative, quantified by the distance a. The charge density determines a parameter λ analogous to the Gouy-Chapman length that quantifies the electrostatic screening length above a surface. A divalent counterion with positive charges separated by a distance δ will aggregate the filament if $\delta < \lambda$, but not if $\delta > \lambda$. From ref. [15].

understanding how biopolymer polyelectrolyte complexes assemble, the structures they form, and what their pathophysiological consequences might be. Such knowledge, based mainly on insights from physical chemistry of electrostatically charged macromolecules, might lead to countermeasures against the pathological consequences of their assembly in vivo.

2. Bundling of like-charged cylindrical biopolymers

2.1. Dependence on filament and counterion surface charge

The mechanism by which like-charged linear polyelectrolytes aggregate into bundles due to interactions with multivalent counterions has been thoroughly studied theoretically by modeling the polyelectrolyte as a charged line with the given linear charge density and approximating the counterions as points or spheres with a given valence. When the linear charge density is sufficiently great that the distance between fixed charges (a in Fig. 1) is less than the Bjerrum length (the distance below which the interaction energy between two fixed unitary charges is greater than kT), a fraction of counterions condense on the linear polyelectrolyte to form a Manning layer and lessen the effective charge density [13]. When the valence and concentration of the counterions exceed a critical value, attractive interactions arise between the filaments. The nature of the attraction is variously described as due to either dynamic or static correlations in counterion spacing that produce local charge inversion and therefore cause the filaments to attract [1•,14]. Most biological polymers have sufficiently large diameters such that their approximation as charged lines is likely insufficient to quantitatively explain why some counterions cause bundling and others do not, even if the general picture of a Manning layer or Wigner lattice mechanism of attraction is qualitatively appropriate. The biological counterions, such as polycationic peptides and steroids, are also large enough potentially not to be treatable as point charges or spheres of given valence. Treating the charged biopolymer filaments as cylinders with curved surfaces introduces a new length scale λ, analogous to the Gouy–Chapman length that quantifies the electrostatic screening length above a surface [15] (Fig. 1). Treating the biological polycations as molecules with defined spacing (δ in Fig. 1) between unitary positive charges also shows that the distance between two positive charges, such as amino groups, can also affect the ability of different polycations with the same valence to bundle biological polymers. A model incorporating these two concepts shows that it might be very useful in identifying conditions at which different filamentous polyelectrolytes are bundled by different biological cations, and can rationalize why divalent cations generally do not bundle DNA whereas they do bundle cytoskeletal filaments [15].

2.2. Effect of counterion structure on bundle packing

The nanoscale structures formed by different biopolymers and different polycations are highly dependent on the length and stiffness of the polymer and the size and valence of the counterions. One recent study showed that double-stranded DNA of fixed length aggregated by the polyvalent peptide protamine formed well-defined aggregates in which the DNA filaments were stretched almost entirely straight and packed with a hexagonally ordered cross-section [16••] (Fig. 2A). The kinetics and final state of assembly of such bundles appear to be controlled by the relative ratios of charges on anionic polyelectrolytes and the cationic counterions as shown in Fig. 2B. When the fixed charges on the electrolyte exceed those on the multivalent counterions, filaments assemble into a range of structures including large organized bundles, single filaments bound to counterions, and filaments without counterions. When the positive charge on the multivalent ions exceeds the negative charge on the polyelectrolytes, the net charge of the resulting polyelectrolyte–counterion bundle can be opposite to that of the free polyelectrolyte. What regulates the steady-state size of bundles is still poorly understood and could result either from kinetic trapping of rapidly assembling large filaments, the effects of the chirality of the filaments, or other geometric features that prevent stiff polyelectrolyte bundles from growing without limit [17,18].

The spacing of filaments within the bundles depends on the nature of the polyvalent counterion, and differences in packing can have large effects on the biological activity of polyelectrolyte bundles. Fig. 2C shows three examples of DNA bundles formed by two kinds of polyvalent cationic antimicrobial peptides (CAPs), LL37 and β-defensin, compared to bundles formed by the trivalent cobalt hexamine [19]. DNA

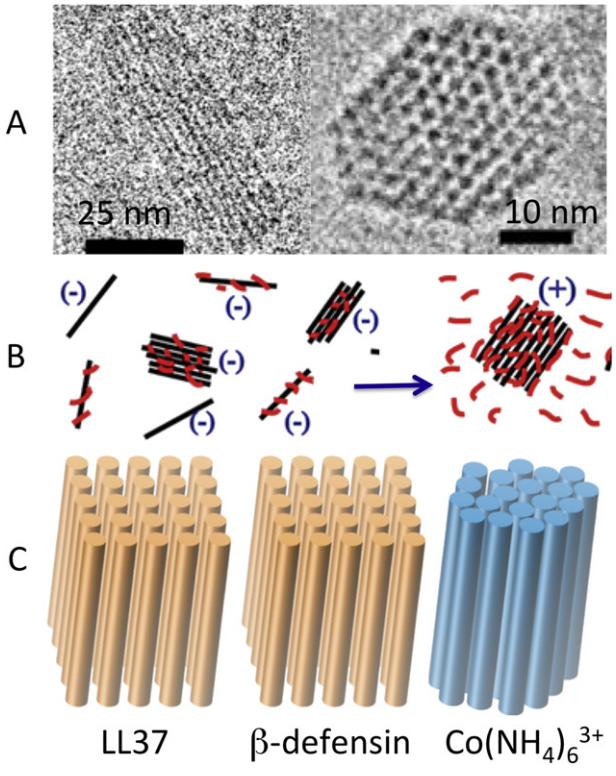

Fig. 2. Structures formed by DNA aggregated by different multivalent counterions. A: Hexagonally packed DNA stabilized by protamine. B: Schematic of reaction intermediate and stable end products when polyelectrolytes aggregate in limiting counterion (left) or excess counterion (right). A and B from [16••]. C: Square and hexagonal packing from different polyvalent counterions, from [19••].