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Zoetic polymers

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Abstract

Conditions mediating the formation of biological polymers in situ are reviewed, and terminology suggested to differentiate polymers found in living cells from synthetic materials and polymers derived from biological sources that are modified or studied in a way that obscures their biological function. Methods currently used to characterize the mechanical properties of biopolymer networks in cells are briefly discussed.
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1. A proposal for new nomenclature

John Ferry's [1] classic monograph on the rheology of polymer solutions and networks had great influence on persons attempting to educate themselves about the methods and findings of a field that lacked an established academic pedigree. Professor Ferry was able to organize and cogently present a bewilderingly large amount of data and mathematical theory, obtained from diverse disciplines such as mechanical engineering, chemistry, physics, and materials science. His book was of enormous assistance to those trying to learn this complicated and fascinating subject, and it significantly helped advance the field to its present active state.

An even more important contribution, perhaps, is Professor Ferry's pioneering work on the mechanical properties of *biological* polymer networks. His studies of the elasticity of fibrin networks, starting in 1947 [2] and lasting for over 40 years [3], showed how classical rheological methods can be used to relate the viscoelastic response of these materials to molecular properties such as polymer concentration, strand length, and intra-strand interactions (e.g., cross-link density). Later work, principally performed in other laboratories but carried out in the same spirit, addressed the mechanical properties of many other polymers of biological origin. Materials that immediately come to mind are actin, tubulin, intermediate filaments, elastin, collagen, mucin, and gel-forming polysaccharides such as agarose. Recent papers that refer to the mechanical properties of some of these materials (e.g., Refs. [4,5]), as well as texts that devote much of their content to the mechanical properties of biological entities (e.g., Refs. [6,7]), attest to the continuing evolution of the subject.

Many of the notions of classical rheology can be carried over when studying the passive mechanical properties of these substances. However, supramolecular biological polymers have properties that distinguish them from many of the materials that traditionally have been studied by mechanical engineers and polymer physicists. Foremost, perhaps, is that the high polymers of biological macromolecules form in aqueous solution, so the properties of the surrounding water need to be taken into account. Due to its polar nature and propensity to form extended structures, and because it interacts with many protein and sugar residues, water almost always is an integral component that has an intrinsic role in the polymerization and interactions of biological polymers. For example, because of the properties of the surrounding water, polymers of actin, tubulin, HbS, and several other proteins actually *de*polymerize at lower temperatures, and

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polymerize into extended strands when the temperature exceeds a critical value. These materials can be cycled between their polymerized and unpolymerized states merely by warming and cooling.

Most supramolecular biopolymer polymerization processes are reversible, in that the bonds that hold the polymer together are not covalent. Even such materials as crosslinked fibrin networks initially form because of noncovalent interactions, to be followed later by enzymatic conversion to a stiffer and more durable material. Another characteristic of biological networks is that, in situ, they form in the presence of other molecular species of similar physical character. Some of these interact directly to crosslink strands or otherwise influence network properties, but others play the role of "crowders" that affect the structure and reactivity of the building blocks of the supramolecular polymer of primary interest [8]. In fact, the protein concentration in the interior of cells can approach 30% [9]. Also, one may need to think in terms of linked, or interpenetrating, networks when considering intracellular phenomena.

The polymerization of the cytoskeletal components, actin and tubulin, into long polymers strongly depends on the presence and rate of hydrolysis of nucleotides, viz., adenosine triphosphate (ATP) in the case of actin, and guanosine triphosphate (GTP) in the case of tubulin. These tubulin polymers, known as microtubules, are stabilized by bound GTP molecules and, when the rate of hydrolysis to guanosine diphosphate (GDP) exceeds the rate of GTP binding, the microtubules depolymerize. The over-riding characteristic of these active polymers is that, within cells, they exist in *dynamic* networks that participate in such essential processes as motility and mitosis. During these events, the networks change in structure (both size and form) due to interactions with regulatory binding partners and/or by phosphorylation or other post-translational modification of their constituents. Moreover, the networks are unlikely to be isotropic, as they often nucleate on specific organelles or at sites on a cell membrane. An additional complication is that polymerization takes place in confined environments. In this case entropic effects, alone, may influence association kinetics and network structure [10]. Several normally compartmentalized polymers, including secretory materials [11] and condensed viral DNA [12], are at such high concentrations that their supramolecular form is akin to that of a glass or quasi-crystal.

Some biological macromolecules polymerize into *closed* structures of regular or semi-regular extent, for which no obvious analogs occur among substances not of biological origin. Examples are the capsids of icosahedral viruses [13], clathrin cages [14], and single-walled tubulin rings [15]. The latter, which can be very regular in shape, arise when tubulin is incubated with certain small peptides or depsipeptides [15]. Virus capsids, which appear as closed shells, also usually have very narrow dispersion in size, but clathrin baskets, which are buckyball-like polyhedra, can assume a relatively wide continuum of sizes depending on environmental conditions. The in vivo assembly of these closed structures is mediated by associations with other proteins. In vitro studies, though, indicate that during assembly the initial addition of subunits to these shell-like entities seems to involve only small changes in free energy, and only the completion of the structures by the last few elements imparts stability. Contacts between the last elements and their neighbors are greater than between subunits early in assembly, but the exact mechanism imparting stability to these closed, finite polymer structures yet needs to be explained. Clathrin heavy chains, like intermediate filament proteins, contain coiled-coil regions [14,16] which may provide flexibility to the individual building blocks that allows them to change their structure slightly as the supramolecular polymers grow.

My interactions with Professor Ferry amounted to only one or two letters, the details of which I've forgotten. I had been working on measuring the elastic properties of fibrin gels [17] by a dynamic light scattering technique and probably communicated my results to him. I recall receiving a very kind note in which Professor Ferry helped me sort out the meanings of various technical terms appearing in the literature. It was important to him to make distinctions that add precision and eliminate confusion. In this spirit I'd like to propose new nomenclature—*zoetic* polymers—for supramolecular biological polymers that have the properties described above. The word "biopolymers" is too inclusive, as it can signify any polymeric material derived from biological sources even if it has been significantly modified, and it fails to distinguish between, e.g., single macromolecules and polymers of macromolecules. Although "supramolecular biopolymer" might capture the idea that many individual building blocks act in concert, the expression is somewhat cumbersome. Both terms fail to capture the notion that certain polymeric forms and activities (e.g., regulated change in structure) are essential to life processes. Unfortunately, one cannot call these entities "living polymers," as that term already is used by the polymer science community to identify polymers whose free ends exchange subunits with building blocks that are free in solution. Although this class includes various biological polymers (notably, microtubules and actin filaments) [18], the designation also has been used for vinyl and other synthetic polymers [19].

The etymology of *zoetic* most likely is the Greek word *zōē*, meaning *life.*

Therefore, zoetic signifies "of, or pertaining to, life." If adopted, though, one might wish to make an additional distinction between "active" and inactive zoetic polymers: it is hard to view agarose, extracted from seaweed and used as a support for electrophoretic separation, with the same awe as actin or tubulin polymers, which appear in eucaryotic cells as dynamic structures having myriad roles in essential life processes. We might exclude agarose from the class of zoetic polymers, except when it functions in a biological

context; similarly, one would leave out "heat-set" gels made up of denatured globular proteins [20], although amyloid fibrils [21] would be included. Of course, one usually can find conditions where potentially active zoetic polymers appear to be fixed and static, in which case classical rheological methods can be used to probe their properties. But those conditions usually do not capture the essence of behavior in a living system and one risks missing some interesting and important science.

2. Measuring the properties of zoetic polymer networks in living cells

As Professor Ferry clearly recognized, a measurement of mechanical properties can be used to explore the underlying molecular organization of an object, even when the latter is very complex. Moreover, since mechanical forces are central to many cellular functions, quantitative values of viscoelastic parameters oftentimes may be needed to explain certain life processes. We here provide a brief overview of recent work that has been carried out to assess the viscoelastic properties of intact, living cells.

One way to sample cell matrix elasticity is to pull on beads that are attached to cell surface receptors that span the plasma membrane and connect to interior cellular components. Wang and Ingber [22] probed the rheological properties of the cytoskeleton with magnetic beads coated with peptides that bind integrins. They were able to demonstrate how the mechanical properties of the cytoskeleton changed in response to changes in cellular environs. However, this magnetic bead rheometer essentially indicates the static elasticity of cells, yet dynamic rheological behaviors frequently are of paramount interest. A related technique, developed by Valberg and Feldman [23], makes use of ingested magnetic particles to directly assess the 'apparent' cytoplasmic viscosity of phagocytic cells. The relaxation of the remanent field from the ingested particles, transiently aligned by an externally imposed magnetic field, provides a bulk measure of cytoplasmic resistance to particle reorientation. However, the inferred viscosity depends on the size of the probe particles (reviewed in Ref. [23]). Moreover, the technique provides a value that is averaged over the entire cell and, because typical particle relaxation times are of the order of several minutes, it is difficult to distinguish kinetic cellular responses.

Other techniques that use beads as markers of cell elasticity involve optical detection. For example, methods based on diffusion wave spectroscopy (DWS) have been developed that enable studies of simple models of reconstituted cytoplasm [24,25], and particle tracking ("multipoint rheology") schemes have been advanced to probe localized regions of the interior of a living cell [26,27]. In both cases, though, many of the aforementioned issues still need to be addressed. An additional factor is that the cell interior may contain extensive pre-stressed networks of interconnected elements, giving rise to nonlinear viscoelastic behaviors [28,29] that are difficult to study by these methods. Moreover, these optical schemes probe the response of individual cells and, although one can study several cells and combine results, it is difficult to characterize the detailed kinetics of rapidly changing cytoskeletal properties by these means.

The need to probe the mechanical dynamics of cytoskeletal change is well illustrated by studies of the response of neutrophils to chemotaxins such as fMLP (*N*-formyl Met–Leu–Phe), a peptide released by bacterial pathogens. When an individual neutrophil is drawn into a small capillary, the protruding end of the cell extends and retracts pseudopods with quasi-periodic motions whose frequencies depend upon the concentration and type of chemotaxin, as well as other environmental conditions [30]. Similar behavior is seen in cell transit time (CTT) experiments, where the resistance to passage of neutrophils through a microporous filter [31] undergoes a transient change after activation by chemoattractants (see Fig. 1). It can be shown that the variation in transit time is directly related to change in the mechanical properties of the cortical actin mesh underlying the plasma membrane [32]. One advantage of this assay is that it probes several hundreds of cells during a single, rapid measurement and therefore is well suited for mapping cell dynamics. The behavior of cells is intrinsically stochastic (due to variations in environment and/or history), so a large sample oftentimes is needed to obtain meaningful information relating to average, i.e., characteristic, response.

Rapid reorganizations of host cell cytoplasm also are involved when certain bacteria, e.g., *Listeria monocytogenes*, move within infected cells [33]. The bacteria are propelled by continuous polymerization and depolymerization of elongated structures formed from actin and other cellular factors. However, the rates of assembly and disassembly of these comet-like tails and, hence, the speeds of the bacteria, depend on the local milieu, confounding the analysis of in vivo motion but affording a means to probe

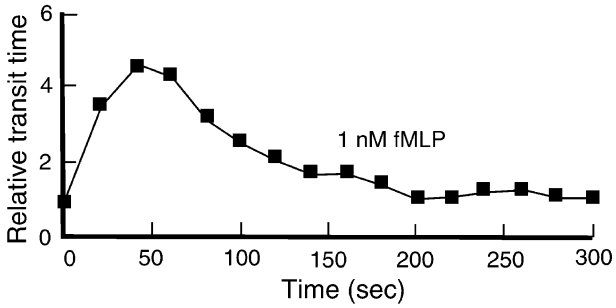

Fig. 1. Relative transit time (TT) of neutrophils pushed through a 20 μm thick microporous filter (8-μm-diameter holes) by hydrostatic pressure, as a function of time after activation with 1 nM fMLP (after Ref. [31]). Each point represents an average over a 20 sec interval. The change in transit time most likely indicates polymerization and depolymerization of the cortical actin layer of the cells [32]. Note the rise and subsequent oscillatory decay of the response, which depend on the concentration of fMLP [31].

the mechanical state of a heterogeneous intracellular environment [34].

Another clever technique, which provides both regional details of the elastic properties of a crawling cell and information about its time-varying response, involves tracking fluorescent beads that have been incorporated into an elastomeric gel used as a substrate for cell locomotion [35]. The gel is distorted and the changing positions of the beads indicate the forces exerted by the cell as it crawls. This method, like the cell transit analyzer [31], gives a somewhat indirect measure of the internal organization of the cell, but nonetheless furnishes data to test kinetic models of cell activation and locomotion. Other promising methods that are being used to assess the mechanical properties of living cells involve atomic force microscopy [36] and laser tweezer technologies [37].

Many of the aforementioned techniques have been developed during the past 15 years and one can expect new advances to be forthcoming at an accelerated pace. Professor Ferry should have been pleased to see how his pioneering studies have led to this vigorously evolving field, which promises to be of increasing significance in cell biology research.

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