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Translational biomedicine-oriented exploratory research on bioactive rotifer-specific biopolymers

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Abstract

There are numerous surprising discoveries in current comprehensive biopolymer research, including the description of new types of biopolymers and the extension of their applications. The discovery of a new rotifer-specific biopolymer family (Rotimers) and the exceptional ability of these micrometazoans to inactivate and catabolize human-type neurotoxic aggregates (e.g., beta-amyloids, alpha-synucleins, prions) by their exudates can be mentioned as the original work of our research group. Rotimers are exogenous and protein complex molecules with a calcium-dependent production mechanism in both bdelloid and monogonant rotifers. However, their experimental and application possibilities are still unknown; only part of the class has been explored and described. Current Rotimer-related studies present promising biodiversity and bioactivity of these biomaterials (e.g., anti- and disaggregation effects or high degrees of adhesion to other molecules). The primary objective of current research is to explore and develop their application in translational biomedicine. A key area is the design of drug candidates against neurodegeneration-related aggregates based on the molecular information provided by the composition, structure and function of Rotimers. These novel biomaterials have the potential to open new perspectives in the pharmaceutical industry and healthcare.

Key words: biopolymer, rotifer, exudate, Rotimer, biomedicine

Biopolymers as molecules of the future

Biopolymers, as organic substances that occur in large numbers and completely degrade in nature, can be applied in several disciplines, from food technologies to conservation procedures, drug carrier-related pharmacology, and in the development of different nanoparticles (e.g., in translational biomedicine).[1] Living organisms produce biopolymers from specific molecular units that can be classified based on their chemical composition, such as polymerized forms of nucleotides, saccharides and peptides, or their complexes.[2] In many cases, stabilizing metal ions, such as magnesium or calcium, are required to form the structure of polymers.[3] These biomolecules (e.g., spider silk, collagen, cellulose, chitosan, albumin, casein) have been studied in various ways, making their extended use unquestionable.[4] However, their molecular properties can vary depending on how the organisms that produce them have changed and adapted to the environment. These agents of biological origin are used in the pharmaceutical and energy industries, as well as in the natural environment.[5] The living organisms involved in biological secretion processes depend on their endemic microenvironment, which has a regulatory role in production of the agents.[6,7] According to the generally accepted theory of evolution, the origin of life is connected with water; thus, the first biopolymer molecules were produced by species living in ancient seas, such as snails, sea urchins and ammonites.[8] The abovementioned biocomposites were soft or hard (or complexes of soft and hard ones), with different protective functions for proto- and metazoa from chemical, ultraviolet and thermal influences.[9] Due to the diversity of natural habitats (e.g., oceans, lakes, rivers, streams, thermal springs, puddles, etc.), microscopic organisms have developed multifunctional biopolymers with properties such as energy and food sources, metabolism and structural constituents, and cell and tissue regeneration functions in the relevant organisms or their environment.[10] These macromolecules exist inside and outside the cells, such as in the extracellular space, cell wall, exoskeleton, or external secretions in soil and water.[1] Natural (ecological) and artificial (laboratorial) niches are extremely sensitive to physicochemical and biological parameters.[11] The formation and production of these species-specific substances can be influenced by several environmental factors, from light intensity to osmolarity, temperature, redox state, and even from dissolved gases to the presence of prey and predators.[12] The increasing utilization of natural materials, such as adapting biodegradable and renewable products, is an essential consideration in the industrial and pharmaceutical sectors, especially due to its environment-friendly approach.[13]

Human-related exceptional properties of rotifers

In several cases of scientific research, one can gain information on specific substances (e.g., organic molecules) from unexpected natural sources (e.g., plants like wheat, soybean, corn, sorghum, rubber trees; animals like sea anemones, shellfish, spiders), with possible retrospective human applications, such as in different forms of medicine.[14,15] The focus has thus shifted from micrometazoans to rotifers, a less explored and studied scientific world. These smallest animals in the world (i.e., eutelic organisms) have been long accepted models for aging, longevity and pharmaceutical studies, or relevant indicators of drug and toxic substances screening, and have even been used in space exploration.[16,17] Due to the widely used omics technologies (genomics, transcriptomics, proteomics, glycomics, and lipidomics), a new alternative model could be employed in basic research and biomedicine; therefore, these phylogenetically ancient rotifers have not yet been investigated in the context of biopolymer production and application of these products.[18] These microscopic organisms are multicellular animals that make up a significant portion of biomass globally.[19] Both bdelloid and monogonant types are found in saltwater environments as well as in freshwater ones.[20] Although their body consists of only approx. 1000 cells, they have independent and complex organs, such as reproductive, digestive and secretory units; moreover, they exhibit extreme resistance to radiation doses and cryptobiosis. In addition to the abovementioned potential, these animals are ideal and promising model systems of special metabolic pathways, thematically connected to human-type neurodegeneration.[21] Rotifer-related research (Fig. 1) presents the interdisciplinary processes in this theme. Our research team was the first to describe a unique and exceptional natural phenomenon, namely the ability of these animal models (e.g., *Philodina acuticornis* or *Adineta vaga*) to degrade and catabolize neurotoxic aggregated peptides/proteins (e.g., beta-amyloids, E46K alpha-synuclein, cellular and scrapie prion proteins), applying it as an exclusive source of material and energy. These Alzheimer's disease-, Parkinson's disease- and prion disease-related human-type toxins were consumed as food and served as special regulating factors to rotifers.[22,23] The tested toxic aggregates did not have the expected and described pathological effects on animals; on the contrary, they increased the lifespan of the treated (i.e., "fed") entities. As opposed to the above, the peptides/proteins proved to be toxic in the viability tests measured on tardigrades and nematodes. Considering the unconventional catabolic ability of rotifers (presumably an evolutionarily evolving property to break down natural debris), the question arises: By what kind of biochemical mechanism can these simple creatures execute the molecular processes described above? This inactivating phenomenon against

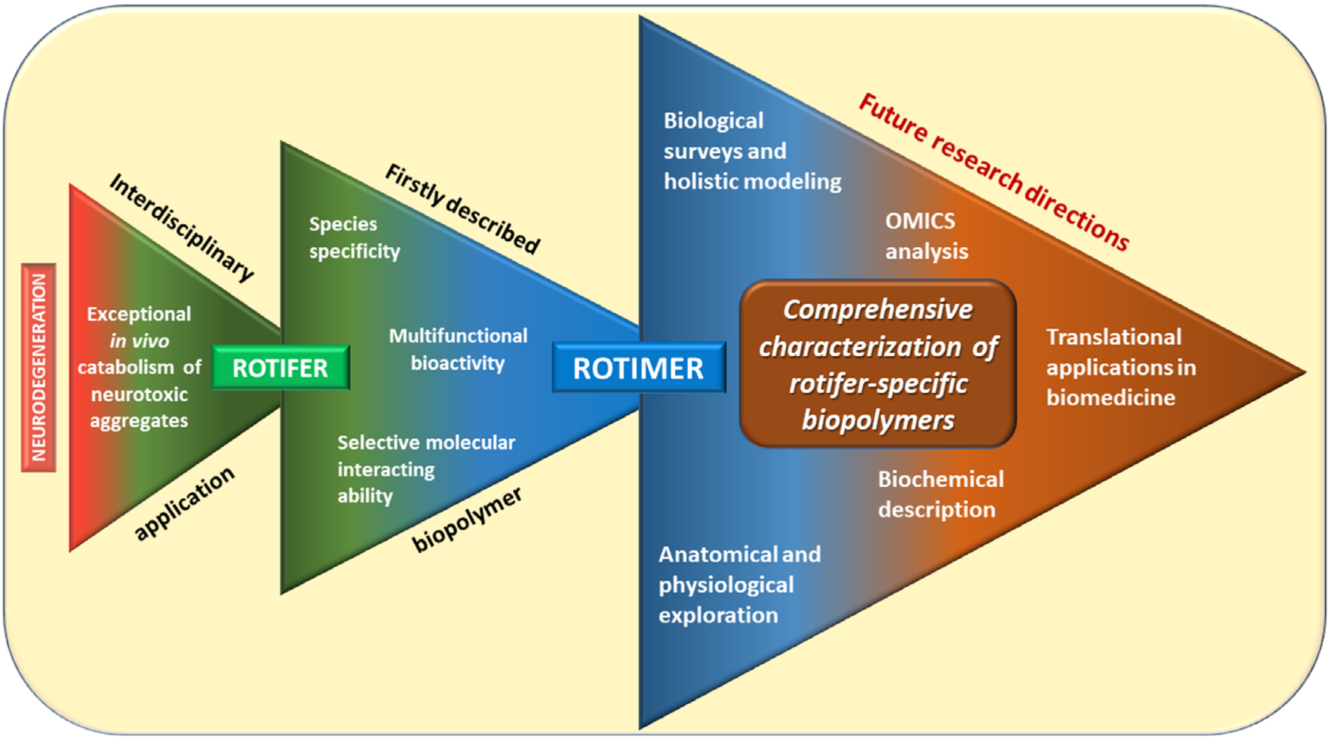

Fig. 1. Exploratory research on newly discovered bioactive Rotimers

notorious neurotoxic aggregates is also remarkable because researchers have not found a solution for neutralizing and degrading these "hard" materials under physiological conditions for several decades. Although rotifers and their species-specific exudates do not directly interact with neurotoxic aggregates in nature, an interaction with specific mechanisms can develop between them under artificial conditions. The autocatabolism–attenuator effect of beta-amyloids in these animals also demonstrates such a case.[24] A similar interdisciplinary regulatory interaction is the effect of kynurenic acid on the metabolism of rotifers.[25] Several studies have proven that this ancient molecule alone prominently and adequately represents the multifunctionality occurring in nature or specific effects on the nervous system.[26–28] However, the relevance between micrometazoa and human studies can only be applied and interpreted to a certain extent. In the innovative intersection of seemingly independent scientific fields, including the topic of biopolymer research, interdisciplinary extensions have no limits, reaching out to physiology of aging or mental phenomena.[29–31]

The Rotimer family and recently discovered biopolymers

While studying the exceptional ability of rotifers and the abovementioned catabolic mechanism, a special exogenous secretion with relatively high tensile strength was observed in several species. The fact that rotifers can secrete filamentous and glue- or film-type bioproducts (Fig. 2) following particle-related mechanical stimulation has recently been discovered and described in the academic literature.[32] These rotifer-specific bioactive

Rotimer characteristics				Rotifers			
				Bdelloids		Monogonants	
				Philodina acuticornis	*Adineta vaga*	*Euchlanis dilatata*	*Lecane bulla*
Conglomerate form	Fibrillar	Floating	Thin	+	+	+	+
			Thick	+	+	+	+
		Fixed	Simple	?	+	+	?
			Double	?	+	+	?
	Amorphous	Gluelike		?	+	+	+
		Film-coated		+	+	?	?
Type	Proteinous	Proteaze-sensitive		+	+	+	+
Function	Bioactivity	*In vitro* (antiaggregation)		?	?	+	+
		In vivo (immobilization)		?	?	+	+

Fig. 2. Species-specific characterizations of Rotimers (+ – known; ? – unknown)

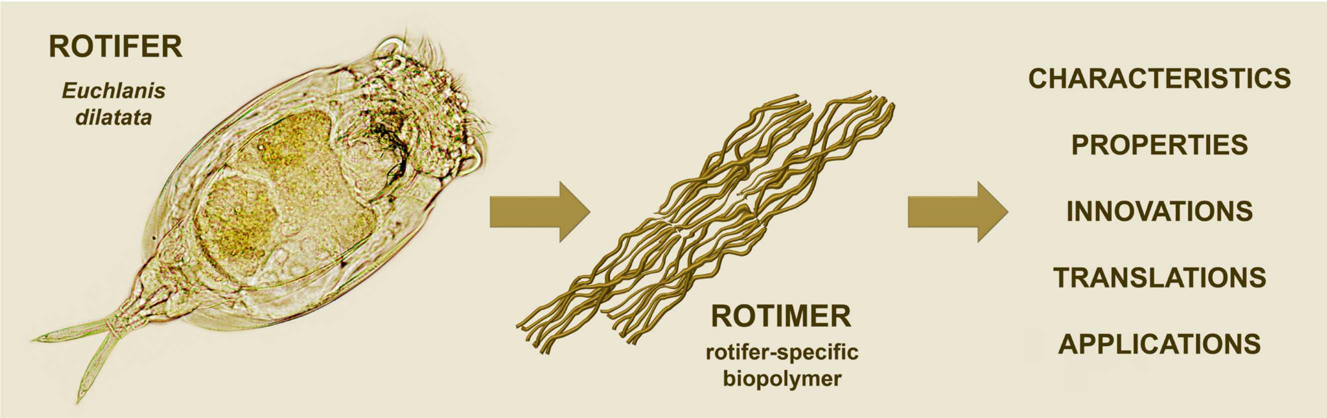

Fig. 3. The Rotifer–Rotimer system and concept

biopolymers, named Rotimers, are multifunctional molecules of these micrometazoans (Fig. 3) that are located inside and outside their bodies. From capturing particle-based food to plucking rotifer eggs (adhesive nature) and cleaning the relevant medium (antiseptic and filtration effect), these tiny creatures use their exogenic exudates in nature. It is known that numerous protozoa, plant and animal species produce polymers, similarly to rotifers. The composition, structure and molecular nature of this novel bioproduct have not yet been explored. Due to its novelty, a complex molecular family of Rotimers potentially holds several yet unknown advantageous properties. However, preliminary measurements have revealed their diverse and promising bioactivity (e.g., inhibition of cancer neuroblastoma cell proliferation and migration, high intercalation and absorption capacity, partial disinfection ability, anti- and disaggregating effects on beta-amyloid aggregates). The induction of Rotimer secretion, both under natural and laboratory conditions, can only be triggered by mechanical irritation of the cilia using different types (e.g., microcrystalline cellulose, urea or carmine crystals, sand, epoxy beads) and sizes (approx. 2–50 μm in diameter) of inert particles as inducers. The external formation of this newly discovered adhesive and protease-sensitive molecule is a calcium-dependent process that can only be observed using optical microscopy when biopolymers and particles form a Rotimer–Inductor Conglomerate (RIC) complex in a random web form. The production of entities and populations by RIC depends on their viability, energy level, and chemical (e.g., toxic agents, osmolarity, pH) and environmental (e.g., temperature) effects. By examining the RIC isolates of several rotifer species, it was found that this conglomerate is resistant to various chemical effects (e.g., detergents, acids, bases, chelators) and has cell immobilization bioactivity with low toxicity.[20] The holistic biological evidence and molecular composition/structure of this protein-type natural biopolymer are unexplored in detail but generally predictable. The use of Rotimers leads to new approaches in biotechnology, pharmaceutical research and biomedicine (e.g., dermatology, neurology).

Possibilities offered by Rotimers in biomedicine

Based on the observation that many freshwater rotifer species can produce different Rotimers, it can be assumed that this biomaterial, a type of biopolymer, is phylogenetically formed and evolutionarily ancient (presumably more than 0.5 billion years old). This proteinous multifunctional macromolecule may be a key component of the abovementioned catabolic and neurotoxic aggregate inactivation capacity.[33] Therefore, the aim of future research is to fully understand the different groups and types of Rotimers, and explore their molecular structure and possible translational and multifunctional applications. The related novel and interdisciplinary methodologies, the phenomenon of catabolism of aggregated proteins, the existence of Rotimers, and their pilot characterization were recently introduced. The research timeline and work on rotifers and their secretions have reached a stage from which forward movement can only happen after gaining knowledge on specific, holistic and molecular endpoints. Investigating the full range and potential applicability of Rotimer biomolecules can shed light on their species specificity, molecular composition and structure, and possible use as active substances. Moreover, these natural products can serve as a good starting point for amassing information about their artificial molecular design and medical applications. The full characterization of this new family of biopolymers provides an exceptional opportunity to launch new projects and to develop further application areas on countless lines. Based on the currently available data, it may be applied indirectly in chemistry (e.g., as a drug carrier) or externally in medicine (e.g., on the skin surface). These special Rotimer exudates are promising new biopolymers that may serve as raw materials for industrial production and more advanced applications, such as developing drug candidates in pharmacological research or translational medicine (e.g., regenerating tissue matrix or even as carriers of active substances).

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