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Ingestion and digestion studies in *Tetrahymena pyriformis* based on chemically modified microparticles

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Abstract

Recognition of food and, in consequence, ingestion of digestible particles is a prerequisite for energy metabolism in *Tetrahymena pyriformis*. Understanding why some particles are ingested and digested, whereas others are not, is important for many fields of research, e.g. survival of pathogens in single-celled organisms or establishment of endosymbiotic relationships. We offered *T. pyriformis* synthetical bovine-serum-albumin (BSA)-methacrylate microparticles of approximately 5.5 μm diameter and studied the ciliates' ingestion and digestion behaviour. Different staining techniques as well as co-feeding with a transformant strain of *Escherichia coli* revealed that *T. pyriformis* considers these particles as natural food source and shows no feeding preference. Further, they are ingested at normal rates and may serve as sole food source. A pivotal advantage of these particles is the convenient modification of their surface by binding different ligands resulting in defined surface properties. Ingestion rate of modified microparticles either increased (additional BSA, enzymes) or decreased (amino acids). Furthermore, we investigated glycosylation patterns by lectin binding. By binding different substances to the surface in combination with various staining techniques, we provide a versatile experimental tool for elucidating details on food recognition and digestion that may allow to study evading digestion by pathogens or potential endosymbionts, too.

Keywords: Ciliates; Digestion; Food vacuole; Ingestion; Microparticles; *Tetrahymena*

Introduction

Recognition of potential food sources and, as a logical consequence, ingestion of food particles, are important prerequisites for many processes in a ciliate cell. A suitable model organism for studying ingestion and digestion in protozoa is *Tetrahymena pyriformis* (e.g. Nelson et al. 2003; Nilsson 1977; Ricketts 1971a). Also selective feeding has been studied in some detail (e.g. Boenigk and Novarino 2004; Thurman et al. 2010). *Tetrahymena pyriformis* seems to depend on particles in ambient media as a stimulus for initiating the formation of food vacuoles. The ciliates' uptake and growth rates in filter-sterilized and consequently particle-free medium are reduced drastically in comparison to autoclaved medium or medium containing particles (Rasmussen and

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Kludt 1970; Rasmussen and Modeweg-Hansen 1973), which recommends this organism for studying the effects of different food particles on ingestion and digestion.

In this study, we offered *T. pyriformis* artificial microparticles with approximately 5.5 μm diameter, based on bovine-serum-albumin (BSA), embedded and stabilized by a methacrylate polymer matrix. These microparticles can easily be stained with different dyes. In addition, the surface properties can be defined by covalent binding of different substances, thus opening wide possibilities for studying effects of chemical and physical properties on the fate of such particles.

Little is known about food recognition in *T. pyriformis*. Filter-feeding ciliates as *T. pyriformis* seem to show less discriminative selective feeding than other free living protists (Boenigk and Novarino 2004; Jacobs et al. 2006). Other studies suggest differing interpretations (Thurman et al. 2010). By an unknown mechanism, these organisms are capable of distinguishing between biotic and abiotic particles, since starved *T. pyriformis* cultures do not ingest colloidal gold when food bacteria are offered in the same medium, whereas gold particles are observed within *T. pyriformis* in the absence of bacteria (Elliott and Clemmons, 1966). Comparable observations were made by Ricketts (1971a) and Seaman (1961), showing that starved cultures of *T. pyriformis* ingest exclusively useful particles. On the other hand, the addition of proteose-peptone-yeast extract medium (PPY) leads to passive ingestion of latex particles (Ricketts 1971a). Furthermore, different substances reveal various efficiency in stimulation of food vacuole formation. Whereas proteins or peptides like proteose peptone or bovine serum albumin, as well as polypeptides or RNA are highly effective in stimulating food vacuole formation, glutamate, low molecular weight substances like amino acid mixtures, polysaccharides or glucose stimulate vacuole formation only moderately. Sodium acetate is completely ineffective (Ricketts 1972). Since the tested substances vary in size as well as in charge, the author concluded that these traits play only a minor role in stimulating food vacuole formation. Further experiments by Rasmussen and Modeweg-Hansen (1973) pointed out that molecule net charge plays only a minor role, since they provided *T. pyriformis* with particles of uniform size, but different net charges (positive, negative and uncharged) and each of the offered particles enhanced growth similarly. Based on these experiments, it seems reasonable to assume a complex food recognition site at the cytostome of *T. pyriformis*, since biotic and abiotic particles are distinguished and different substances influence uptake rates.

Studies on selective feeding of other free living protists reveal more details about reception mechanisms (Montagnes et al. 2008). Different lectins may be involved in food recognition and binding in *Oxyrrhis marina* (Wootton et al. 2007) and *Hartmanella vermiformis* (Venkataraman et al. 1997). Allen and Dawidowicz (1990) identified a mannose-binding receptor protein involved in attaching and internalizing yeast by *Acanthamoeba castellanii*. How *Tetrahymena* distinguishes between different food particles before ingestion remains unknown. Also a complete phagosome proteome of *T. thermophila* reveals no clues for food perception prior to ingestion (Jacobs et al. 2006). To identify structures involved in food perception, we performed in vivo lectin labelling of *T. pyriformis*. Glycosylated lipids and proteins are often involved in recognition processes, but are also important as target sequences for transport, self- and non-self-recognition and as protection against self-digestion. Therefore, we used the lectins concanavalinA (conA) and wheat-germ-agglutinin (WGA), coupled with a fluorescent dye. WGA binds to terminal, non-reducing N-acetyl-glucosamine and to sialic acid (Wright 1984), which are both found in N- as well as O-glycosylated proteins. ConA labels internal and terminal, non-reducing α-D-manno-pyranosyl- and α-D-gluco-pyranosyl-residues as well as α-D-glucose and α-D-mannose (Goldstein et al. 1974).

The digestion process of *T. pyriformis* was studied in some detail (Nilsson 1977; Nilsson 1987; Nilsson and van Deurs 1983). After food vacuoles are formed and detached from the cytostome, the vacuoles are acidified, which is an important prerequisite for lysosome fusion (Nilsson 1977). In *Paramecium* the acidification is realized by fusion of acidosomes with the food vacuole membrane, a process, resulting in integration of specialized proton pumps (Ishida et al. 1997). How acidification is realized in *T. pyriformis* remains unclear, but vesicles of unknown function formed at parasomal sacks were observed to fuse with early food vacuoles (Nilsson 1987; Nilsson and van Deurs 1983). A phagosome proteome analysis of *T. thermophila* identified three subunits of a membrane associated, vacuolar ATPase that is possibly involved in acidification (Jacobs et al. 2006). After acidification lysosomes fuse with food vacuoles. The maximal acidic pH of 3.5–4.0 is reached after 1 h. Before defecation of food vacuole contents, they are neutralized. The whole digestion process requires about 2 h (Nilsson 1977).

Since ingestion alone is not inducing digestion, an additional recognition system seems to act inside food vacuoles. By measuring acid phosphatase activity as marker for digestion, Ricketts (1971a) showed that food vacuoles containing indigestible material, i.e. latex particles, do not result in increased acid phosphatase activity, whereas vacuoles containing bacteria, yeast or PPY exhibit increased acid phosphatase activity. Similar findings were made by Boenigk et al. (2001) with nanoflagellates. Food vacuoles, containing indigestible particles, were egested 2–3 min after uptake by *Spumella* and *Ochromonas*, whereas vacuoles containing bacteria remained longer within the cells. This points to selective digestion behaviour and consequently to recognition sites within food vacuoles.

Understanding the process of digestion in detail is essential for studying why and how some ingested bacteria are able to evade digestion and in consequence survive or even divide within protists. Especially pathogenic microorganisms were shown to survive the digestive process in *T. pyriformis* and even to propagate inside the cells (Barker and Brown 1994). Some of them lyse the host after propagation or induce