Protist Behavioral Responses to Micromolar Levels of Dissolved Amino Acids

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Introduction

A recent study found that μM β-dimethyl sulfoniopropionate (DMSP), an algal osmolyte, inhibited protist grazing of algae (Strom et al. 2003). Since DMSP is a nontoxic compatible solute, we hypothesized it was acting as a chemical signal. Subsequent examination of chemically related compounds found surprising inhibition of grazing by μM levels of some amino acids, such as proline, serine, alanine, valine, and cysteine (Strom et al., this symposium, **poster #TS24-77**). Although some protists can detect low levels of amino acids as chemosensory cues (Levandowsky et al. 1984) and likely use such signals to detect and exploit food patches (Fenchel & Blackburn, 1999), inhibition of feeding has not to our knowledge been reported. What is the mechanism of grazing inhibition by these common, nontoxic compounds? Are they acting as signal cues, or disrupting normal swimming and feeding behavior?

In this study, we report preliminary analysis of the swimming of algivorous ciliates in the presence μM dissolved free amino acids (DFAAs).

Questions

1. Do DFAAs that inhibit grazing cause a change in swimming behavior?
2. How do we quantify swimming motions? Will bulk average parameters be sufficient, or does analysis need to be on a track-by-track basis?

Methods

Experimental Setup

1. Initial tests used a 15 °C water bath to maintain temperatures (CSP-B1, B2; **table 1**); later experiments were conducted in a walk-in incubator.
2. Ciliates (*Favella*) or dinoflagellates (*Gymnodinium, Oxyrrhis*) were grown on maintenance prey mixtures, then starved 12-24 h.
3. Cultures were dispensed into 100-ml cell culture bottles, and amino acids were added to final concentration of 20 μM. Controls received equivalent volume of distilled water. Some experiments utilized replicate treatments, while others utilized multiple amino acid additions. In some experiments, prey cells were added and grazing rates determined (not shown).
4. Swimming was filmed at time 0, 10, 20, or 30 min for 2-3 min intervals with darkfield microscopy. 30 fps video was generated with a Sony B/W camera and recorded by VCR. Video was digitized with a Videum 1000 frame capture board (Winnov Corp.), and compressed using Intel Video R3.2 format.

Track analysis

1. LabTrack 2.0 (Bioras, Inc.) was used to track cell motion and generate Excel output files. Typically, only tracks >2 s long were retained. Pixels were converted to μm from slide micrometer measurements. Output data included t, X,Y, for each frame, and V, A, and angle averaged successively over 6 frames.
2. Bulk track statistics were calculated with a PERL script. For each track, we calculated the average velocity and 1-s averaged net-gross-displacement ratio (NGDR), a measure of turning rate.
3. Individual tracks were analyzed by wavelet analysis (www.aavso.org/data/software/winwwz.shtml) for swimming patterns. Frequency-amplitude spectra were created and then thresholded to determine helical swimming.

Table 1: Behavioral experiments

Experiment	Objective
CSP-B1	Time course of qualitative behavioral response of *Favella* to proline over 150 min.
CSP-B2	Quantitative behavioral response of *Favella* to chemicals shown to result in varying degrees of feeding inhibition. THIS POSTER.
CSP-B3	Time course of effect of proline on *Favella* grazing. Grazing inhibited through 240 min, though slightly reduced after 100 min. No sign of ciliate mortality.
CSP-B4	Behavioral response of dinoflagellates (*Oxyrrhis, Gymnodinium, Glenodinium*) to addition of proline or DMSP.
CSP-B5	Feeding response and behavioral response of *Favella* to added serine and arginine at 0, 4, 8, 12, and 24 hr.

Results

a. Ciliates swim in helical tracks that differ qualitatively with treatment

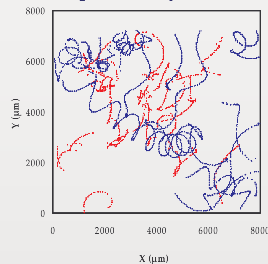


Fig. 1: 100 s of serine t 10s (blue) or alanine 20s (red) paths, showing representative helices. Only ~10% tracks analyzed are shown for clarity

b. Average NGDR and velocity do not differentiate treatments

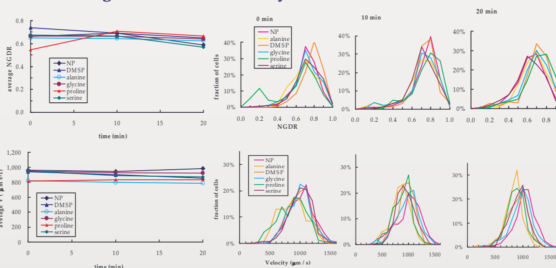


Fig. 2: Average NGDR, Velocity (left) for different treatments. NP = nanopure (control). Right: histogram distributions. Low NGDR for proline t0 is due to noise, not real tracks.

c. Analysis of individual tracks shows contrasting behaviors

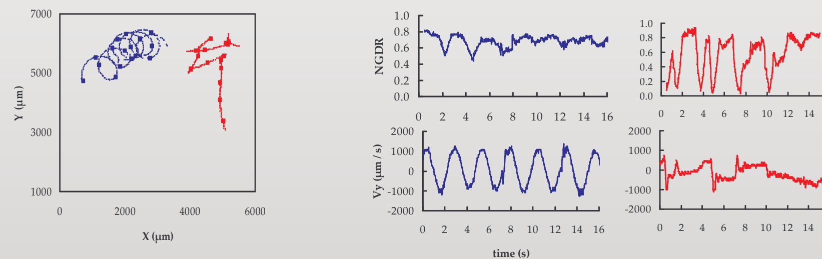


Fig. 3: Examples of helical (blue) and straight (red) tracks. Squares indicate 1 s intervals.

Fig. 4: NGDR (top) and Vy (bottom) for helical and straight tracks in fig. 3.

d. Wavelet analysis can derive statistics of helical vs. straight tracks

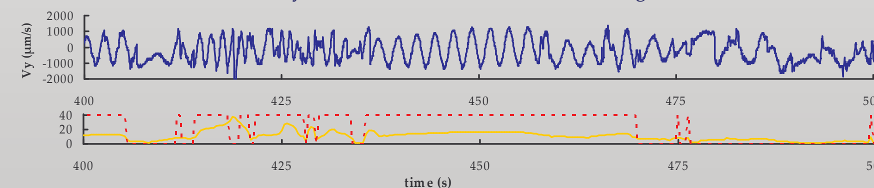
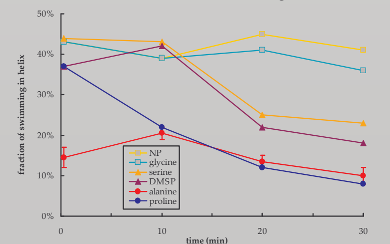


Fig. 5: top: serine t10 Vy for 100 s of cell tracks. Bottom: wavelet analysis of tracks. Yellow line is product of frequency * amplitude. Red dashed line is thresholded result, indicating sinusoidal (helical) motion.



← **Fig. 6:** fraction of cell tracks with helical motion for different treatments, as determined by wavelet analysis shown above. Different compounds inhibit helical swimming to differing degrees, and effects appear to be time-dependent. Glycine shows no significant inhibition compared to nanopure control (NP). Alanine causes immediate cessation of helical swimming, while proline inhibition does not occur immediately, but after 10 min. Serine and DMSP both show effects after 20 min, but are less inhibitory. Error bars for alanine treatment indicate precision of pseudo-replication using Vy and Vx as independent tests of helical swimming. Rotational frequencies did not change significantly over this experiment (not shown).

Discussion

1. Ciliate swimming patterns are typically 3D helices (fig. 1), thought to optimize search behavior (Bartumeus et al., 2003). However, amino acids and analogs that reduced grazing appeared to qualitatively alter normal helical swimming behavior.
2. Average parameters such as velocity or NGDR generally could not adequately detect subtle changes in swimming motion (fig. 2), although there was some indication that alanine and proline caused reduced swimming speeds.
3. This is because in all treatments, a spectrum of cell activity includes both helical and straight-line swimming (figs. 3-4). Per-track analysis is necessary to differentiate these behaviors. Wavelet analysis is a promising method for detecting swimming frequencies, and suggests that ciliates have distinct behavioral responses that varied with amino acid side chain structures (figs. 5-6).
4. Amino acids that strongly inhibit grazing (proline, alanine) appear to reduce helical swimming, at least transiently (fig. 6). Other compounds that also affect grazing (DMSP, serine) have more subtle effects.
5. Future work will refine quantitative analyses of swimming behavior. We also plan to test 2 possible mechanisms by which amino acids and analogs may affect behavior: interference with membrane ion channels, or with membrane stretch receptors.

Summary

1. **Analysis of 3D helical swimming from 2D data is difficult (Crenshaw, 1996, Crenshaw et al., 2000). 3D motion analysis (Thar et al., 2000) is one possible solution.**
2. **However, signal processing techniques (wavelets, Fourier transforms, autocorrelation) may help detect changes in individual cell behaviors.**
3. **With such analyses, swimming responses correlate with grazing reduction, suggesting that the inhibition of feeding is due to changes in swimming behavior that reduce prey interception and capture. A working hypothesis is that amino acids and analogs interfere with ciliate plasma membrane ion channels, causing swimming reversals and other aberrant behaviors.**

Acknowledgements

We thank the following people for their contributions:

Hans Jakobson and Susanne Menden-Deuer, WWU, video and track analysis

Nick Blackburn, Bioras Inc., LabTrack software

Cecily Wolfe, Univ. Hawaii, and Terry Kiser, CSUC, data analysis

Alan Raetz, CSUC, PERL programming

This project is funded by NSF OCE-0325025.

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