INTRACELLULAR pH IN SYMBIOTIC CNIDARIANS

Fundamental physiology in an era of global change

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Overview: We present first-ever measurements of intracellular pH (pHi) in a reef coral and symbiotic anemone. Our approach used a pH sensitive probe and confocal microscopy.

Significance:

Intracellular pH (pHi) is a fundamental parameter that affects all physiological processes. In reef corals, pHi plays a key role in the supply of inorganic carbon (Ci) for photosynthesis by symbiotic algae (*Symbiodinium*) and also to the site of calcification. Despite its importance, pHi has never been characterised in a cnidarian.

Figure 1. Polyp of the reef coral *Stylophora pistillata* (image by E Tambutté)

Coral calcification and photosynthesis are vunerable to environmental changes in temperature and seawater pH, and greater knowledge of coral physiology is needed to understand why. The development of *in vivo* coral cell imaging techniques and determination of pHi are significant steps towards an improved understanding of coral cell biology.

Methods:

Preparation of cells:
Isolated endodermal cells were prepared from *S. pistillata* and *A. viridis* maintained at CSM (Bénazet-Tambutté et al. 1996), suspended in filtered seawater (FSW) and loaded into perfusion chambers. Viability staining (Live/Dead Viability Kits (Invitrogen, CA, USA) confirmed cells remained viable in perfusion chambers for at least 4 hours in FSW. All pH measurements were performed within 90 min after loading into chambers.

Determination of intracellular pH:
Cells were loaded with the pH sensitive dye SNARF-1 AM (Invitrogen) in FSW. Cell analysis was conducted using a Leica SP5 confocal microscope equipped with UV and visible laser lines. Excitation of SNARF-1 was at 543 nm and emission captured at 585 and 640 ± 10 nm whilst simultaneously monitoring in transmission. Measurements of intracellular pH were performed by obtaining the ratio (R) of fluorescence intensity of SNARF-1 AM at 585/ 640 nm with in a region of interest within host cell cytoplasm (figure 5). *In vivo* pH calibration in the range pH 6 -8.5 was performed based on the method of Thomas et al. (1979) (fig. 2). Autofluoresence from algal chlorophyll could be detected within the 640 nm channel (Red in fig. 3), but solely within algal cells and not within host cytoplasm.

Determination of cell ultrastructure:
Cells were examined by transmission electron microscopy (TEM) and confocal microscopy (fig. 3). Nuclei and mitochondria were visualised by staining with Hoechst 33342 and Rhodamine.

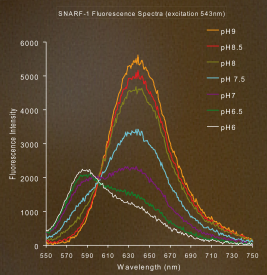

Figure 2. The pH sensitive shift in the ratio of emission at 585 and 640 nm of SNARF-1 when excited at 543 nm FSW.

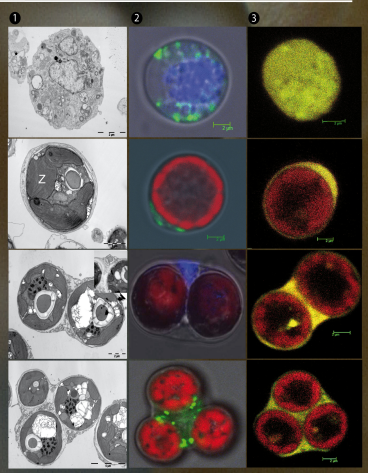

Figure 3. Cells types isolated from *A. viridis* and *S. pistillata*. Columns: 1. TEM images, 2. Confocal images of cells stained with Hoechst 33342 and Rhodamine, 3. Confocal images of cells loaded with SNARF-1 AM. Rows: a) Symbiont-free endodermal cells, b) endoderm cell with one alga (Z), c) with two algae, d) with three algae. In TEM image C1 a food vacuole is shown in the inset. Colours in confocal images represent the following: Red = autofluorescence of chlorophyll in intracellular algae, blue = nuclei stained with Hoechst 33342, green = mitochondria stained with Rhodamine 123, yellow = cnidarian cytoplasm stained with SNARF-1.

Results:

pHi in coral and anemone cells:

1. Values of pHi in coral and anemone cells were more than a pH unit lower than the surrounding seawater pH of 8.1 (see Table 1).

2. pHi values in *A. viridis* cells containing algae and algal-free cells were not significantly different, nor were pHi comparisons between *S. pistillata* and *A. viridis*.

3. When cells were perfused with FSW containing 20 mM NH_4Cl an increase in pHi was observed (Fig. 4). Cells recovered pHi to resting levels 10 min after washing with FSW.

Spatial distribution of pHi:

4. pHi was relatively stable (± 0.1 pH) throughout the depth of the cell in areas associated with maximium SNARF-1 fluorescence in the cytoplasm (Fig. 5).

5. The principle source of spatial heterogeneity in pHi was the immediate area surrounding the symbiotic algae (fig. 6). This area was often observed to be lower than pH6, below the limits of our calibration. Acidic vacuoles were also sometimes observed in coral and anemone cytoplasm, that may correspond to food vacuoles observed by TEM (Fig. 3).

Table 1. Mean pHi values observed in cnidarian endoderm cells.

	Intracellular pH	
Species/ cell type	Mean (n=20)	SD
S. pistillata with symbionts	7.06	± 0.22
A. viridis with symbionts	6.9	± 0.21
A. viridis no symbiont	6.86	± 0.22

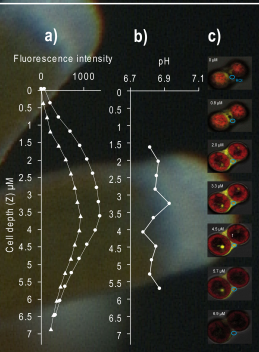

Figure 5. Z (cell depth) profile of an *A. viridis* endodermal cell. a) Fluorescence intensity at 585 (circles) and 640 nm (triangles). b) pHi with cell depth. c) Representative Z stack slices. pHi was determined within the region of interest (ROI) shown (blue).

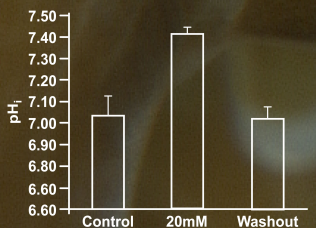

Figure 4. The impact of NH_4Cl on pHi (mean ± SE) in endoderm cells in FSW isolated from *A. viridis*.

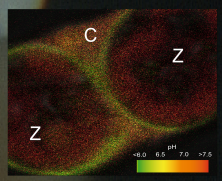

Figure 6. Ratiometric image of SNARF-1 emission (585 and 640 nm) displaying a pH gradient in *A. viridis* endoderm cell. C= animal cytoplasm. Z= Algal symbiont. Note: Red= algal autofluorescence and is not pH related.

Conclusions:

1. Symbiotic corals and anemones maintain a low pH relative to the surrounding seawater.

2. Cells that contain algal symbionts and symbiont free cells have identical pHi.

3. The principle source of spatial heterogenity in pHi in coral and anemone cells is associated with the area surrounding the algal symbionts. This area of low pH may be associated with the symbiosome membrane complex.

4. Our *in vivo* cell imaging approach provides a platform for future work on the impact of environmental change (e.g. low seawater pH) on pHi and other fundamental aspects of coral biology.

References:

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