Characterization of a carbonic anhydrase involved in coral calcification

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Introduction

Carbonic anhydrases (CA) play an important role in biomineralization (calcification) from invertebrates to vertebrates. We have cloned, sequenced and localized a CA from the reef building (scleractinian) coral *Stylophora pistillata*. This CA was named STPCA. Since in corals, calcification rates vary between light and dark, we have determined the expression of the STPCA gene in the dark and in the light. We have also measured STPCA activity and its inhibition by a series of inorganic anions and sulfonamides.

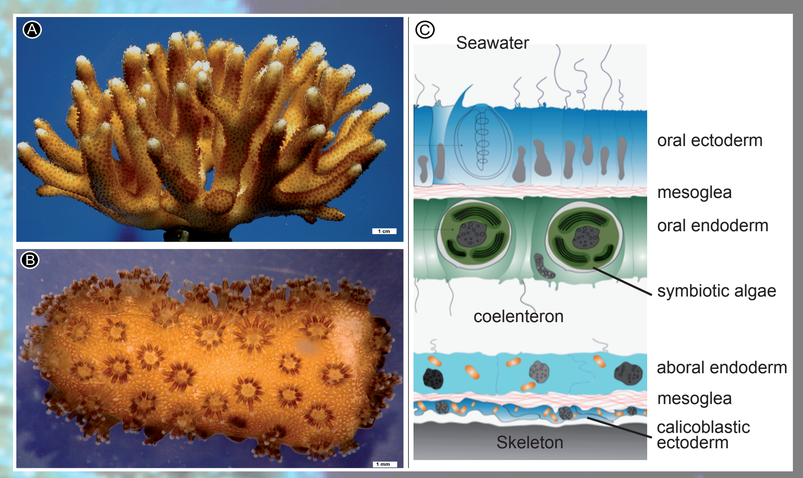


Figure 1 (right): The scleractinian coral *Stylophora pistillata*.
A) Mother colony. B) Microcolony used for physiological studies. C) Schematic representation of the coral histology.

STPCA is a secreted form of alpha-CA.

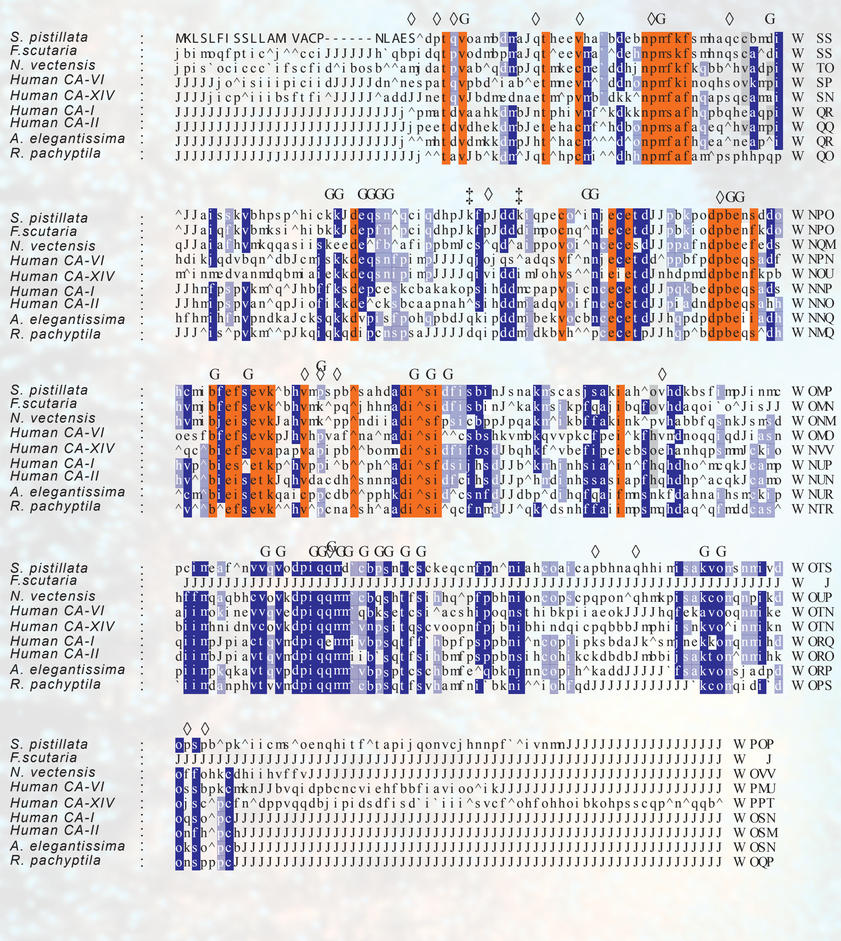


Figure 2 : Alignment of alpha-CA amino acids sequences from *S. pistillata* (STPCA), *F. scutaria*, *N. vectensis*, *H. sapiens* CAVI, XIV, I, II, *A. elegantissima* and *R. pachyptila*.
The first 23 residues of STPCA are a signal peptide. Conserved residues are in orange, 80% and 60% identical residues are respectively in dark blue and light blue. H = Histidines involved in Zinc cofactor binding. Asteriks = residues involved in the hydrogen-bound network. Putative N-glycosylation and phosphorylation sites are shown as ‡ and losenges respectively (Moya *et al.* 2008).

Its catalytic function is similar to the secreted human CAVI.

Isozyme	activity level	Kcat (s^{-1})	Kcat/Km ($M^{-1}.s^{-1}$)	Ki acetazolamide (nM)
hCAI	moderate	$2,0.10^5$	$5,0.10^7$	250
hCAII	very high	$1,4.10^6$	$1,5.10^3$	12
hCAVI	moderate	$3,4.10^5$	$4,9.10^7$	11
STPCA	moderate	$3,1.10^5$	$4,6.10^7$	16

Table 1 : Kinetic parameters for the CO_2 hydration reaction catalysed by the human isoforms CAI, CAII, CAVI and STPCA at 20°C and pH 7.5 in 10mM HEPES buffer ; and inhibition data with acetazolamide (5-acetamido-1,3,4-thiadiazol-2-sulfonamide) (Moya *et al.* 2008).

STPCA is localized in the calicoblastic ectoderm, which is responsible for coral calcification.

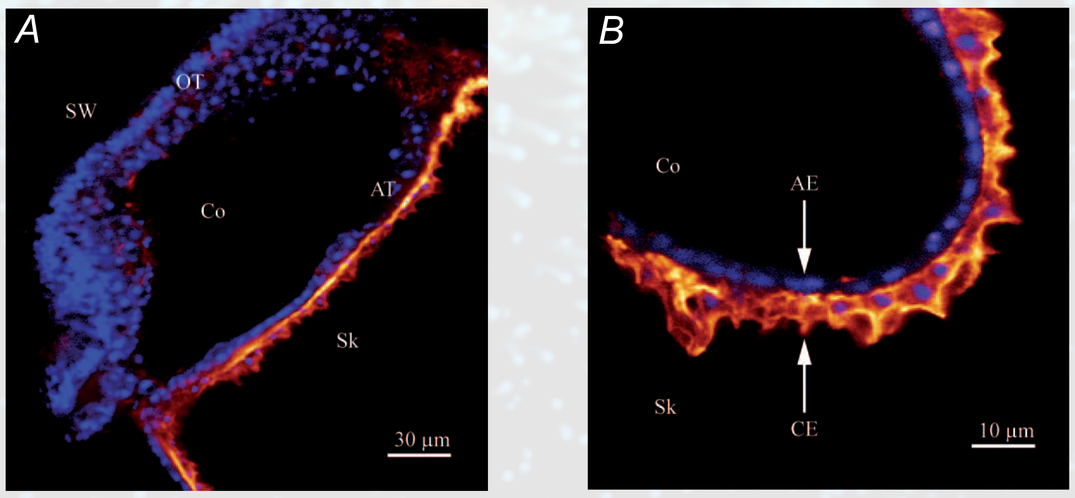


Figure 3 : Immunolocalization of STPCA on a tissue section of *S. pistillata*.
A: view of the oral tissue (OT) and aboral tissue (AT). B: magnification on the aboral tissue showing the aboral endorm (AE) and the calicoblastic ectoderm (CE). SW = seawater ; Co = coelenteron ; Sk = skeleton. Orange : anti-STPCA coupled with Alexafluor 568. Blue : nuclei stained with DAPI (Moya *et al.* 2008).

Inhibition by anions confirms similarities with the human secreted CAVI and indicates that the calcifying medium is different to seawater.

Inhibitor	Ki (mM) hCAI	hCAII	hCAVI	STPCA	Concentration in seawater (mM)
F^-	> 300	>300	0.60	0.62	
NO_3^-	7	35	0.76	0.56	
NO_2^-	8.4	63	0.82	0.77	
Sulfamide	0.31	1.13	0.07	0.010	
Cl^-	6	200	0.72	**0.50**	**546**
SO_4^{2-}	63	>200	9.9	**0.91**	**28**
Br^-	4	63	0.73	**0.0097**	**0.84**
HCO_3^-	12	85	0.80	**0.45**	**2**
CO_3^{2-}	15	73	0.69	**0.010**	**0.2**

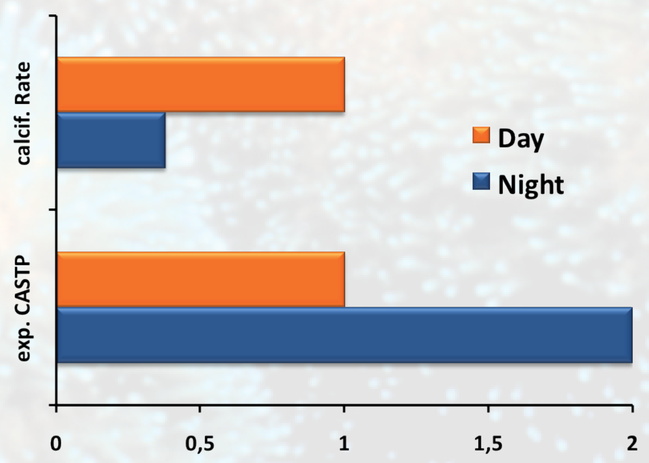
Table 2 : Inhibition constants of anionic inhibitors against human isozymes CA I, II and VI - and STPCA for the CO_2 hydration reaction at 20°C. For details about the full dataset, see Bertucci *et al.* 2009a.

Inhibition by sulfonamides / sulfamates reveals STPCA specific inhibitory responses.

Inhibitor	Ki (nM) hCAI	hCAII	hCAVI	STPCA	
Dichlorophenamide	1,200	38	79	431	
Sulpiride	12,000	40	0.8	430	
Saccharin	18,540	5,950	935	40.3	**Potentially useful for *in vivo* studies**

Table 3 : Inhibition constants of some of the 37 tested sulfonamides / sulfamates.
Stopped flow CO_2 assay method, pH 7,5, 20mM Tris-HCl buffer. For details about the full dataset, see Bertucci *et al.* 2009b.

The STPCA gene is over-expressed to regulate pH and maintain a basal rate of calcification during the night.



Calcification decreases at night as photosynthesis of symbiotic algae stops.
This results in an acidosis in calcifying tissue.

The over-expression of STPCA allows calcification to continue and counters pH variation at the calcifying site.

Figure 4 : Variations of calcification rate and expression of STPCA between the day and the night.
Diurnal values are reported to 1. Calcification was measured by ^{45}Ca incorporation in the skeleton. Gene expression was determined by real time PCR (Moya *et al.* 2008).

Conclusion

This work coupled up molecular, biochemical, physiological and pharmacological techniques to provide the first example of such a complete study of a purified carbonic anhydrase in corals.
This results, combined with experiments on living organisms, will help us to better understand the calcification mechanism in corals and especially the role played by STPCA.

References:
Moya *et al.* (2008) J. Biol. Chem. 283, 25475-25484.
Bertucci *et al.* (2009a) Bioorg. Med. Chem. Lett. 19, 650-653.
Bertucci *et al.* (2009b) Bioorg. Med. Chem. 17, 5054-5058.