



Isolation and Expression Analysis of Three Types of α -Carbonic Anhydrases from the Antarctic Alga *Chlamydomonas* sp. ICE-L under Different Light Stress Treatments

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Abstract

Carbonic anhydrases (CAs) are a class of zinc-containing metalloenzymes that can reversibly catalyse the hydration reaction of carbon dioxide. Antarctic algae are the most critical component of the Antarctic ecosystem; algae can enter the carbon cycle food chain by fixing carbon dioxide from the air. In this study, the complete open reading frames (ORFs) of *CA1* (GenBank ID KY826431), *CA2* (GenBank ID KY826432), and *CA3* (GenBank ID KY826433), encoding CAs in the Antarctic ice microalga *Chlamydomonas* sp. ICE-L, were successfully cloned using reverse transcription-polymerase chain reaction (RT-PCR). In addition, the expression patterns of CAs under blue light, under UV light, and in the dark were determined by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). The *CA1*, *CA2*, and *CA3* ORFs encode proteins of 376, 430, and 419 amino acids, respectively. Phylogenetic analysis revealed that all amino acid sequences showed high homology with those of *C. sp. ICE-L*. There are six types of algal CAs; we hypothesised that the CAs studied here are most likely α -CAs. Expression analysis showed that the transcription level of the CAs was influenced by both UV light and blue light. These findings provide additional insight into the molecular mechanisms of CAs and will accelerate the development of CAs for applications in agriculture and environmental governance.

Keywords *Chlamydomonas* sp. ICE-L · qRT-PCR · Bioinformatic analysis · Carbonic anhydrase

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Introduction

Carbon is an essential element for all life on earth, and it is the basis of all biological organic compounds [1]; however, higher carbon dioxide (CO₂) content in the air is unfavourable. Based on estimates, prior to the industrial revolution, the volume of carbon dioxide in the air was only 280×10^{-6} , in 2011, the CO₂ concentration in the air has reached 391×10^{-6} , an increase of 40%, and continues to increase at an annual rate of approximately 2×10^{-6} [2]. As the CO₂ content in the atmosphere increases, the CO₂ partial pressure rises and the ocean absorbs more of it. CO₂ dissolves in water and ionizes to produce H⁺, leading to a reduction in the pH of seawater; ocean acidification is progressively becoming a serious problem and will eventually threaten some marine life [3, 4]. To solve this problem, it is necessary to reduce the CO₂ concentration in the air. Scientists have been examining several ways to reduce CO₂ concentrations such as solvent absorption, adsorption, CO₂ membrane separation, cyanobacteria, and biological transformation [5–7].

Initial methods were energy-intensive and produced waste streams that were unfriendly to the environment. Subsequently, investigators started to realise the advantages of CO₂ bioavailability methods, such as the use of algae and cyanobacteria, for capturing CO₂ [8, 9].

Tsai et al. [10] found that algal cells exhibit a higher rate of CO₂ uptake and consumption than other plants; algal cells can accumulate a large amount of CO₂ in carbon sinks in a relatively short period of time and allow its entry into the carbon-sink food chain. The carbon sink function of algal cells is inextricably linked to the role of carbonic anhydrase (CA) [11]. CAs were first discovered in mammalian erythrocytes and have subsequently been identified in most organisms [12]. To date six distinct classes of CAs, namely α , β , γ , δ , ζ , and η , have been reported [12, 13]. These six types exhibit low amino acid sequence similarity, do not share any obvious homology, and have different active site structures, suggesting that these six proteins have evolved via convergence [13, 14]. Moreover, these six enzymes are distributed in different cells, tissues, and organelles, and algal cells can either indirectly transport HCO₃⁻ into the cytosol through the plasma membrane and then convert HCO₃⁻ into CO₂ by intracellular CA, or they can directly convert HCO₃⁻ into CO₂ via the extracellular periplasmic CA catalytic cell surface and then distribute it into the cell [6, 15–17]. The extracellular and intracellular activities of CA aid the key enzyme ribulose carboxylase/oxygenase (Rubisco) to maintain the concentration of CO₂ in the cells, so that algae cells can sustain high photosynthetic efficiency in lower CO₂ environments [6].

Although all six classes of these enzymes possess CO₂ hydratase activity that catalyses the immobilisation of CO₂, their catalytic activities vary [16, 18]. The catalytic activity of α -CA is highest, followed by β -CA and η -CA, while the catalytic activities of γ -, δ -, and ζ -CA are the lowest [16, 19]. In addition to CO₂ hydratase activity, α -CA has esterase activity that catalyses ester hydrolysis; the other five CA types do not possess esterase activity [16]. Many organisms express more than one type of CA, and eukaryotic algae contain most types. Animals and humans express α -CA and β -CA [20, 21]; α -CA is distributed throughout nearly all mammalian tissues and participates in various processes in the body [21]. α -CA, one of the earliest discovered CAs, was first purified from bovine erythrocytes [20, 22]. The active site metal ions of these CAs differ: α -, β -, δ -, and possibly η -CA require zinc ions in the active sites of the protein; ζ -CA can use either Cd²⁺ or Zn²⁺; and γ -CA can utilise either by Zn²⁺, Co²⁺, or Fe²⁺ [12, 13, 23–25].

CAs not only catalyse carboxylation and decarboxylation reactions, but they also participate in the inorganic carbon transport processes of algae and plants; they transport inorganic carbon from highly respiratory cells, transport inorganic carbon into strongly photosynthetic cells and promote

CO₂ to 1,5-diphosphate Rubisco diffusion enhanced photosynthesis in algae cells. In addition, the CA activity of *Chlamydomonas* sp. ICE-L is affected by light at different wavelengths [26].

The CA present in *C. reinhardtii* is the most studied among eukaryotic algae. Previous studies have identified 12 CA-encoding genes in *C. sp. ICE-L*, including three α -CAs, six β -CAs and three γ -CAs [27, 28]. Most α -CAs have a molecular weight of approximately 30 kDa. In this paper, we report the isolation and expression analysis of three types of α -CAs from *C. sp. ICE-L* under light stress treatments, which may provide reference values for further studies on CO₂ concentrating mechanisms (CCM), carbon metabolism, photosynthesis, and other physiological processes of polar algae, especially the Antarctic ice algae *C. sp. ICE-L*. These findings will lay the foundation to elucidate the adaptation of polar algae to extreme environments, and thus, to apply of genetic alterations to economic crops in order to improve their adaptability to adverse conditions.

Materials and Methods

Algal Isolation and Culture Method

The Antarctic ice alga *C. sp. ICE-L* was isolated from floating ice near the Zhongshan Research Station of Antarctica (69°S, 77°E) [29, 30]. The sample was maintained at the Laboratory of Marine Bioactive Substances, the First Institute of Oceanography, State Oceanic Administration, China. Cultures were grown in Provasoli-enriched seawater with an optical density of 40 μ M photon m⁻²s⁻¹, under a 12/12-h light dark cycle at 5 °C [29].

RNA Extraction and cDNA Synthesis

Total RNA was extracted using TransZol reagent (TransZol, TransGen Biotech, Beijing, China) according to the manufacturer's instructions. RNA integrity was determined by electrophoresing the sample on a 1% agarose gel, while RNA quantification and purity were determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). cDNA synthesis was performed using the EasyScript One-Step gDNA Removal and cDNA Synthesis SuperMix according to the manufacturer's protocol. The resulting cDNA was stored at -20 °C or -80 °C until further use.

Cloning and Sequencing of CA cDNA

In order to obtain a large number of amplified target gene fragments, specific primers were designed based on potential full length cDNAs, which were obtained from the transcriptome of *C. sp. ICE-L*. The PCR products were separated by

electrophoresis on 1.0% agarose gels, and the desired fragment was excised and purified using the EasyPure PCR Purification Kit (TransGen Biotech). The target products were then cloned into the vector, pMD-18T (Takara Bio, China). The cloned and propagated plasmids were sequenced at Sangon Biotech (Qingdao) to determine the sequences of the cloned cDNA fragments.

Transcriptional Regulation of CAs Under Different Stress Conditions

Logarithmic phase algae cells grown in normal culture were subjected to different stress conditions to study CA mRNA expression. Algae were exposed to 400 μM photons $\text{m}^{-2}\text{s}^{-1}$ of UVB (Beijing Normal University, Beijing, China), red-light (Shanghai Yongkang, Shanghai, China), blue light tube (Shanghai Yongkang, Shanghai, China), and darkness for 0.25, 1, 2, 3, 6, and 8 h. In addition, algae cells were cultured in normal light for the same interval set, as a control experiment.

Bioinformatic Analysis

Multiple-sequence alignments of CAs were performed with the CLUSTAL X program [31]. Open reading frames (ORFs) were predicted using the National Center for Biotechnology Information (NCBI) Web site (<https://www.ncbi.nlm.nih.gov/orffinder>). The homology of the sequences was examined using the BLASTX function of the NCBI Web site. The nucleotide sequences of the CAs were translated into protein sequences (<http://searchlauncher.bcm.tmc.edu/seq-util/Options/sixframe.html>) and compared with the NCBI protein databases (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). A similarity matrix based on multiple-sequence alignments of different translated amino acid sequences was analysed using the DNAMAN program. Then, protein subcellular localisation was analysed using prediction tools (<https://www.genscript.com/wolf-psort.html>), and the theoretical isoelectric point (pI) and molecular weight (Mw) of each target protein were calculated with the Compute pI/Mw tool of ExPASy ProtParam (<http://web.expasy.org/protparam>). Finally, Mega6.0 software was used to generate a phylogenetic tree based on the neighbour-joining method and evaluated by a bootstrap analysis with 1000 replicates [32].

Quantitative Real-Time PCR (qRT-PCR) Analysis

CA-specific primers (Table 1) were designed using Primer Premier 5.0 software. The housekeeping gene, GAPDH (Table 1), was used as an internal control. qRT-PCR was performed with an ABI StepOnePlus™ Real-Time PCR System (Applied Biosystems, USA) and SYBR Premix Ex

Table 1 Primers used in qRT-PCR

Primers	Sequence (5'-3')
GAPDH	
Forward	GCAGCGACATCCCTACCT
Reverse	CCCTGACAATGCCGAAC
KY826431	
Forward	ACATCACAAGGAACTCAGCCATACG
Reverse	GGTCAGGACGAACCAATCCACAC
KY826432	
Forward	GATGATTGGAGTGGAGTGGATGGC
Reverse	CGTTGGAGATGAGACTTGGATAGGAC
KY826433	
Forward	GCCAAGAACGCAGACCCGAGTG
Reverse	CCGTCGCCTTGATGGTGTTAGTC

Taq II (TaKaRa Biotech Co., Dalian, China). The qRT-PCR amplification conditions were as follows: 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, 58 °C for 15 s, and 72 °C for 30 s. The experimental data were further analysed using the Ct ($2^{-\Delta\Delta\text{CT}}$) model [33]; to ensure consistency, the baseline was automatically set by the software. All algae samples were tested in triplicate in parallel.

Results

Cloning and Sequencing of CA Genes

The ORFs of CAs were identified from the transcriptome of *C. sp. ICE-L* and amplified by semi-quantitative RT-PCR with specific primers. The sequences of the CAs were submitted to GenBank and assigned the following accession numbers: KY826431, KY826432 and KY826433. A comparison of the amino acid sequences of the *C. sp. ICE-L* CAs with other CAs is shown in supplementary Fig. 1.

Bioinformatic Analysis

The sequence of CA1 was 1131 bp in length and encoded a polypeptide of 376 amino acids (Fig. 1a) with a theoretical pI of 8.85. The sequence of CA2 was 1293 bp in length and encoded a polypeptide of 430 amino acids (Fig. 1b) with a theoretical pI of 5.80. The sequence of CA3 was 1131 bp in length and encoded a polypeptide of 376 amino acids (Fig. 1c) with a theoretical pI of 9.16. The Mws of the CA proteins were 40.33 kDa, 46.81 kDa, and 41.11 kDa, respectively. Multiple sequence alignments constructed using (predicted) CA proteins from *C. sp.*, *C. reinhardtii*, *Green algae*, and *Dunaliella salina* (Supplementary Fig. 1) and indicated that the identify of CA1, CA2, and CA3 equalled to 54.77%, 51.23%, and 53.61%.

Fig. 1 Nucleotide and deduced amino acid sequences of three cloned CAs: **a** CA1, **b** CA2, and **c** CA3 from the Antarctic Alga *C. sp. ICE-L*

a

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1 M K L N L T S S Q S A V A R S R A P A A R G V K L A P A R R N V S V S
1 ATGAAGCTTAACTTAACATCTTCGCACTGTGAGTCCGCGGATAGGCGCCAGCTGCCCAGGCGTTAAGCTCGCTCGACCAATGTCTCCGTCAGT
36 A L P P A E S E A N D A L A E T I S R K V K P M L V S D L L N V P K L L
106 GCCTCCCTCCTGCCGAGAGCGAAGCCCAAGCATGCCTTGGCTGAGACAATTTCCGCCAAGGTTAAGCCCATGCTAGTCTCGGACCTCTGAATGTGCCTAAGTTG
71 K R R R G L M M A G A A A M F G C E C C S D M L G T G A A Q A N A S W T
211 AAGCGAAGGGGTTGATGATGCGCGGGCGAGCCGCTATGTTGGATGCGAATGCTGCAGCGACATGTTGGGCGCCGAGCTGCCAGGCAATGCTAGCTGGACC
106 Y K E D F S G P D F W P D Q C L T G G A Q S P I D L S L A N N R P Q
316 TACAAGGAGGACGGATTGACGGGACCTGACTTCTGGCCTGACCACTGCTTACTGGGAGCCGACCTCCCCATCGACTGTCCCTGCCAACACCCGCCCCAG
141 A G R G T I K F P V G P L Q F D Y G R Y E K V N V L N T G H T M Q V
421 GCAGGACGGGTACCATTAAAGTTCCCGTAGGCCCTTCAATTGACTACGGCGGTACGAGAAGTCAACGTGTTGACACTGGCCATGGAACAAATGCAAGTGC
176 N F Q P G N M V T V P K R Y V F V R P P S Q L L A S S P G G A C L C P
526 AACTCCAGCTGGCAACATGGTACGGTCCCAAGCGTTAGTACCTGCGCCACCGCTCAGCTTACGACGCTCCCGAGGGGGCGCTGTTGCGCCC
211 T K P F D L D L I Q Y H F H T P S E H A I D G K Q A M E A H L V H R
631 ACCAAGCCTTTGATTGGACCTTATTCAGTACCATTCCACACTCCAGTGAGCATGCCATTGATGGAAGACAGGACGATGGAAGCACATCGGTGCATCGG
246 N K N T G G L A V V A V L I Q P G G P Q P N P C L E L G L E E A P T E
736 AATAAGAACCGGGTGGACTTGGCTGCTGGCTGTGCTCATCAACCTGGAGGGCCCGAGCCCAACCCGCTTGGAGTAGCTTTGAAGAGGCTCCCCAGAG
281 G G V Q Q A S T K T V N P A M L L P R A T S Q G T Q P Y V H Y T G S L
841 GGTGGTTCACGAGCCCTCGACAAGACCGTGAACCCGCGCATGCTGCTCCCGGGCAACATCAAGGAACCTCAGCCATGACCCGACTACAGGCTCTCTT
316 T T P P C S E G V D W F V L T T P I K V P D K Q V L D F M R F A G N G
946 ACCACTCCCGCTTGTCTGAGGGTGTGGATTGGTCTGCTGACACGCGCATCAAGGTCCCTGACAAGCAGGTGCTTGAATTCATCGCCTTGTGCAATGGA
351 K G Y V R N S R P L Q P I N G R E I D Y S D Q F Q I *
1051 AAGGCTACGTGCGCAACTCCCGACCCCTGACGCCATCAACGGCCGTGAATCGACTACTGACCAATTCCAGATTTAA
    
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b

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1 M C G H V L L I L G V V L A L A A S E A C V Y K Y G T S P G S E A P
1 ATGTGGCCATGACTGCTGATTCTGGGGTGGTGTGCTGACTGGCTGCCGCGAGTGAGGCATGTGTGACAAATACGGGACATCCCTGGTAGTGAGGCCCC
36 S V S G T S W D Y K V M G D D W S G V D G H N K S W S C M S G L H Q V
106 TCCGTGAGTGAACAGGTTGGACTACAAGGTGATGGGGATGATTGGAGTGGAGTGGATGGCCACAAAGTCTGGTCAATGATGAGTGGGCTGCCACAGAGT
71 P I D L P V S A H I G D P S L I S K W S Y P S L I N G S N I E V I N
211 CCCATTGACCTCCCTGTATCTGCTCATTGCTGACCCAGCCTTATTTCCAAATGGTCCCTTCCAGTCTCATCTCCAAAGGATCCAATATCGAGGTCATCAAC
106 N G H A I Q V E W T S N Y E G S M D I V A D A S P A A T I T D I L S M
316 AATGGACATGCAATCCAGGTAGAATGGAGCTCTAAGTACGAGGGTCCATGGACATCGTGTGACGCCCTCACCAGCCGCTACCATACCAGACATTTTGCATAT
141 P D T S E G T K V V T A R P L Q F H F H T G S E H F M D G D A L A M E
421 CCAGATACCTTGAAGGCCCAAGTGGTGGAGCGCCGCGCTCGAGTTTCACTCCACACTGGGAGTGAGCATCTCATGGATGGTGATGCTTGGATGGAG
176 M H I V S R V P H S E M P G C P E A G C L T V T A V L F K V R E G P C
526 ATGCATATCGTGAAGCGTGTACTCATTAGAGATGCGAGGCTGTCTGAGCTGGATGCTGACAGCTGTTGATGTTAAGTGGAGAGGAGGCGCCCTG
211 N A A L K D L F T S L P M E E G T I N F L S E G A T I D L G K V L P S
631 AACGCTGCTTAAAGGACTTGTTCACGAGCTGCCATGGAAGAGGGCACCATTAATTTTGGTGAAGTGTACCATGATTGTTGAGGAGGATGCAACAGC
246 K E K R S Y Y Q A Y A G S L T T P P C S E G L L W H V L D E A S H S W
736 AAGGAAAAGCGCTCTACTACCAATATGCTGTAGCTGACCACTCCTCCGCTGTTGGAGGGCTTCTTGGACGCTCTTAGACGAGCCAGCCAGCCACATCAGCTGG
281 K Q Y R A L L M A V S D K D C T L K A E D T S K V P R A S T G Q M Q Y
841 AAGCAGTACAGGCACTACTAATGGCAGTCAAGGACTGACCCCTCAAGGCTGAGGACACAAGCAAAGTCCCAAGAGCACTACTGGCCAGATGCAATAC
316 L R S S G S L Q N L H R S H T A Q R A L R S L S S S E G M P Q Y L E
946 TTGGCTCTTCCAGGATCTCTCCAGATCTGCACAGGCTCTACTAGTGCACAGAGGGCTCTAGCATCATATATCTTCCAGAGGGATGCTCAGTACCTGGAG
351 V V V S E L K S L Q R D R Q G V L V L Q S S A V L V L T A G S D I N K I
1051 GTGGTGGTTCCGAGCTAAAAGCCCTACAAGGGATCGCCAAGGGGCTTGGTATTGAGAGCTCTGCTGTAGACACCTGCTCCTGGATCCGATTTGAGAGG
386 H K D T Y V C V A Y A Y S Q N R R L P Q P S N D R M I K Y F E A S K E
1156 CACAAGACACATATGCTGTGTAGCTTATGGTCTACTCCAGAATCGCCGCTGCCAGCCATCAAATGACAGAATGATCAAATATTTGAAGCAAGCAAGAG
421 S D M I A E A I K A *
1261 AGTGATATGATAGCAGAGCTATCAAGGCGTAA
    
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c

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1 M L L S K G S S S S T L S R N P Q R S S R Q F S H L E Q L L R R S N G
1 ATGCTGCTTTCARAGGATCTTCCATCCACGCTGTCGCGGAATCCCCAACGTTATCGCGCAATTTCCCATCTAGAGCAACTGCTCAGCGGTAGCAATGGA
36 Q V A G V K T S S S N R D E V T P L E G A A G E A S K P Q L I T K L V
106 CAGTTTGGGGGTAAGACCTCATCATTAATCGGATGAAGTGAAGCCACTAGAGGGTGGCGGGGGAGGCTTCCAGGCCCAATTTGATACGAAGCTAGTT
71 R E P N K I K R R G L M M A G A A A M F G C A C C A D L S S A K A N
211 AAGGAGCAAAAGATTAAGCGAGGGGCGTATGATGGCAGGTGCTGCAGCCATGTTGCGATGCGCATGCTGCGCGGATGTTATGTCGTCGGCGAAGCCAA
106 E W G Y G G E A P A S W P G V C T T G G R Q S P I N V T L A P K I K S
316 GAGTGGGTTACGGTGGAGAGGCCCCGCTTCTGGCTGGAGTGTGCACCACTGGAGGGGGCAATCAACCCATTAATGTACACTGACACCTAAGATCAAGTGC
141 A Q G K T K Y P V T P I K F N Y G R R E K V E V L N T G H T M Q V N
421 GCACAGGGGAGACCAATACCACTGACACCGATAAAATCAACTACGGGGGCGCTGAGAGGTGGAGTCTTAAACACTGGGCGAGCCCAATGCAAGTAAAT
176 F K A G N S A Y V P P R F I P S D E V S A Y P G D E V S E Q N A G P C L C
526 TTCAAGGCGTAACCTGCTCAGTCCCGCCCGCTTATCCCATCTGATGAGTGTCTGCTCCCTCCGTTGGGATAGCGAACAACCGCCGCTTGCCTCTGT
211 P S K P M D L E L I Q Y H F H T P S E H Y L D G E Q T I M E A H L V H
631 CCCTCCAAACCCATGGACTAGAACTCATCCAGTACCCTTCCACAGCCTAGCGAGCACTCTAGATGGTGAACAGACACCATCATGGAGGACAGCTAGTCCAC
246 R N R Y T G G L A V I A V L I R S G G S T P N P C L Q L G L D F G P E
736 CGGAACCGCTACACCGGGACTAGCGGTATAGCTGCTCATCCGCTCAGGGGGAAGCACCCCAATCCATCCAGCTCAGCTAGGGTGGATTTGGGCCAGAG
281 E S G R R V P A R R A A N P Y L L P A K N A D R V Q P Y V H Y Q G S
841 GAGAGTGGGAGCTGTACCGGCAAGCGGGCCGCAATCCCTATCTCCTGCTACTGCCAAGAAGACGAGCCGATGCAACCCGATGTCGACTACAGGCGCTG
316 L T T P P C S E S V D W F V M T N T I K A T D K Q V I D F M R Y S G G
946 CTCACGACCGCCGCTGCACTGAGAGCGTGGACTGTTGTGATGACTAACACCATCAAGGCGAGCAGCAGGCTATTGACTTATCGCCTACTCAGGAGCC
351 G F G Y K Q N S R P V Q E M N A R M V E Y F E Y D I *
1051 GGCTTTGGCTACAAGCAGAACCGCCGCGTGCAGGAATGAACGGCCGATGGTGGAGTATTTTGGATGATGATTTGA
    
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Moreover, CA1, CA2, and CA3 were located on the chloroplast, cytoplasm, and chloroplast predicted by protein sub-cellular localisation. Following the successful cloning of the CAs, the ORFs were predicted using NCBI ORF Web site. A phylogenetic tree was constructed using MEGA 6.0 (Fig. 2). Next, the tertiary structures were reconstructed using ExPASy, as shown in Fig. 3. It was predicted that CA1 (Fig. 3a) and CA3 (Fig. 3c) are homo-dimers and that CA2 (Fig. 3b) is a monomeric structure, based on the research of Waterhouse et al. [34]. The protein transmembrane domains were predicted using TMHMM version 2.0,

and the results, including inside-to-outside helices and outside-to-inside helices, are shown in Table 2.

qRT-PCR Analysis of CA Expression

The relative mRNA expression results are presented in Fig. 4. The expression of CAs in algal cells differed in response to UV and blue light stress. Under UV-light, all three CAs were obviously upregulated within the first 2 h and then rapidly decreased until 6 h, when gene expression levels were lower than those of the control group.

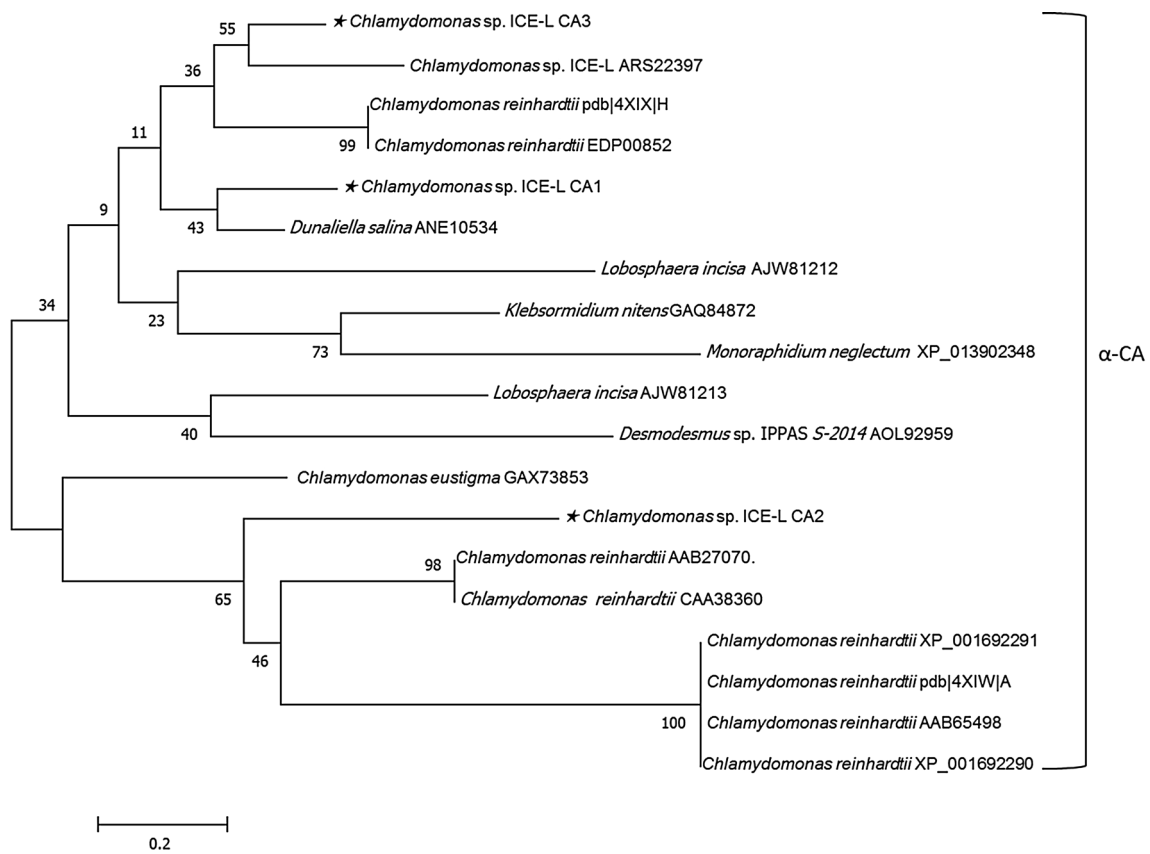


Fig. 2 Phylogenetic analysis of CA proteins. Phylogenetic tree based on the neighbour-joining method and evaluated by bootstrap analysis with 1000 replicates. GenBank accession numbers used to construct the tree were as follows: *C. sp. ICE-L CA1* (KY826431); *C. sp. ICE-L CA2* (KY826432); *C. sp. ICE-L CA3* (KY826433); *C. reinhardtii* (pdb|4XIX|H, EDP00852, AAB27070, CAA38360,

XP_001692291, pdb|4XIW|A, AAB65498, XP_001692290) *Dunaliella salina* (ANE 10534); *Lobosphaera incisa* (AJW81212; AJW81213); *Klebsormidium nitens* (AQ84872); *Monoraphidium neglectum* (P_013902348); *Desmodesmus* sp. IPPAS (S-2014 AOL92959); *C. eustigma* (GAX73853)

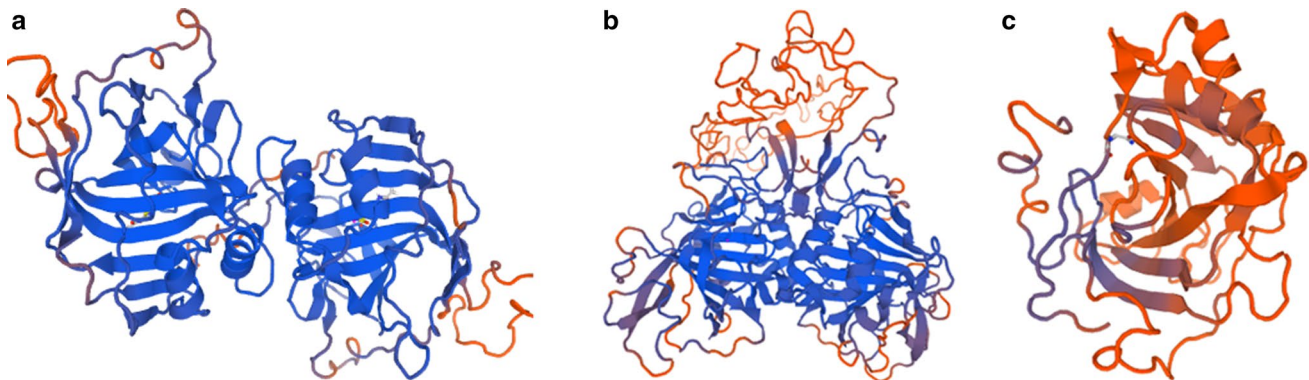


Fig. 3 Tertiary structure fabrication of CAs: **a** (KY826431), **b** (KY826432), and **c** (KY826433)

Similarly, under dark conditions, CA expression reached its highest value at 2 h and then began to decrease. The response was slightly different under blue light conditions; the *CA1* and *CA2* genes showed the highest level of mRNA expression at 3 h, while the *CA3* gene reached its highest

level at 1 h. Interestingly, under the stress of UV light stress, the expression levels of these CAs were relatively high, compared with those under red light and darkness, and the lowest gene expression occurred under blue light conditions.

Table 2 Possible transmembrane helical regions

	Inside-to-outside helices				Outside-to-inside helices			
	From	To	Source	Centre	From	To	Source	Centre
CA1	74 (74)	99 (93)	1065	85	74 (74)	93 (93)	548	84
	248 (248)	264 (264)	353	256				
CA2	5 (5)	24 (24)	1785	14	4 (4)	21 (21)	1793	13
	120 (122)	140 (138)	61	130	187 (187)	203 (203)	125	195
					254 (254)	272 (272)	42	262
CA3	80 (80)	102 (99)	1747	91	80 (80)	99 (99)	1416	90
	249 (251)	267 (267)	733	259	249 (251)	267 (267)	489	259

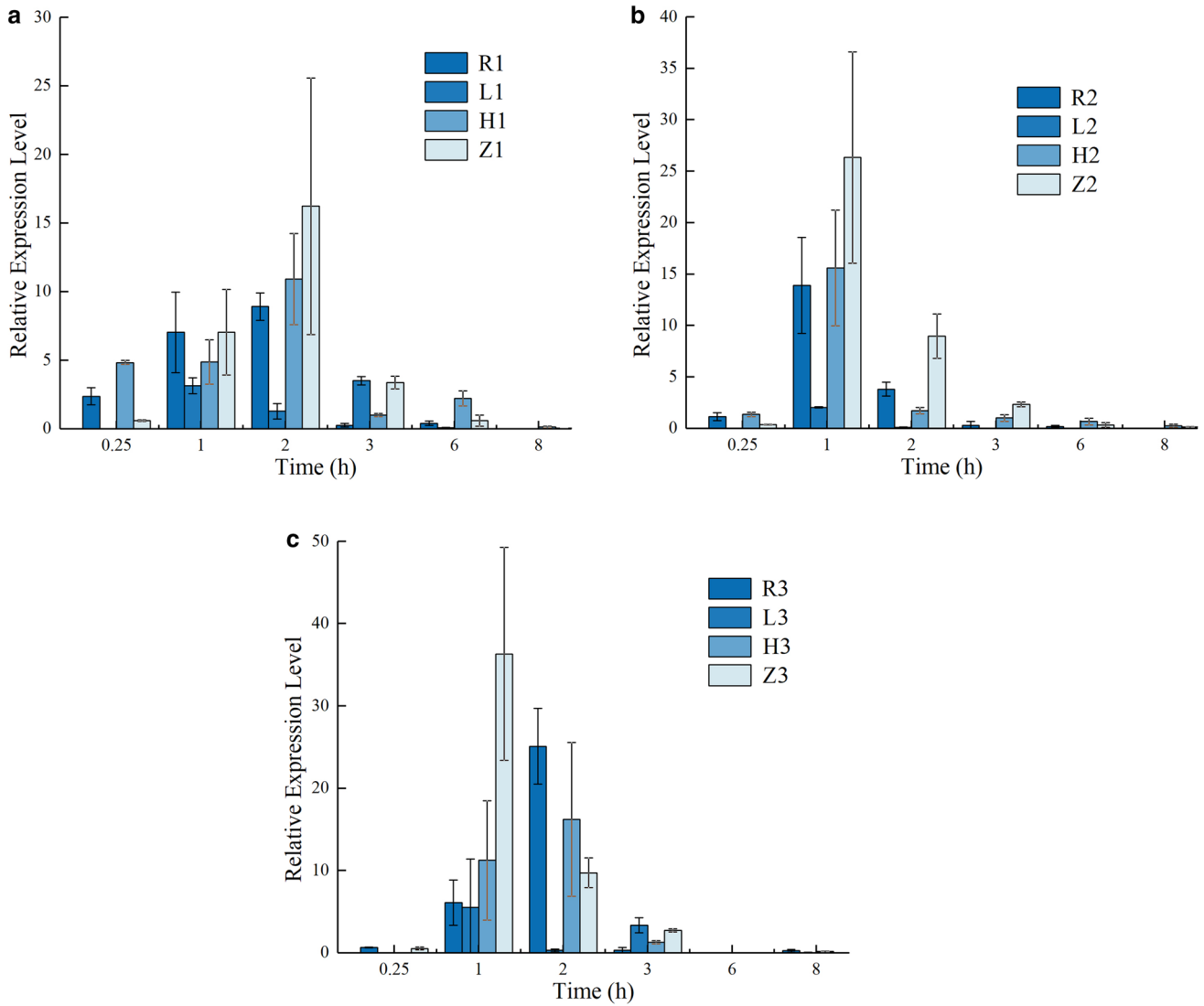


Fig. 4 Relative mRNA expression of CAs in algal cells in response to different conditions. Standard error bars are shown. Each sample was assessed in triplicate (R-red light, L-light, H-dark, Z-UV light, **a** KY826431, **b** KY826432, **c** KY826433)

Discussion

In previous studies, we found that CAs are present in vertebrate erythrocytes and in many tissues of various animals and plants. Moreover, the functions of the CA family have been widely reported, especially in terrestrial plants and animals [35, 36]. Many photosynthetic organisms, especially eukaryotic algae, can transport CO₂ to the plasma membrane, chloroplast envelope, and by CAs, which are one of the most important parts of the carbon-concentrating mechanisms [37]. Thus, those CAs can contribute to reducing in the concentration of CO₂ in the air to mitigate the greenhouse effects. In Antarctica, the low temperature and stronger UV radiation negatively affect of most living things producing a fragile environment. Thus, the presence of Antarctic algae has a significant stabilising effect.

This paper reports three CAs obtained from *C. sp. ICE-L*. Bioinformatic methods were utilised to analyse the encoded proteins to predict their tertiary structures and transmembrane domains in order to further understand their function and mechanism of action. Through analysis, we found that CA2 localises to the chloroplast, which were similar to the CA as reported by Qu et al. [38] CA1 and CA3 localised to the cytoplasm. We speculated that the different function of these CAs may be depended on the different localisation in the algal cell. CA1 and CA3 were predicted as dimers, and their tertiary structures were similar to those of Benlloch et al. [39].

In addition, CA activity can be induced by a certain intensity of blue light, whereas visible light has no activating effects [26]. Antarctic solar UV radiation is intense. Thus, we considered the effects of different light stresses on CA expression. The three types of CAs have different responses. There was a similar pattern between CA2 and CA3. Specifically, their relative mRNA expressions reached a maximum of approximately 25 and 37, respectively, after exposure to UV light for 1 h. Regarding CA1, it took 2 h to reach its maximum. The upregulation of CAs with UV light exposure suggests that they participate the adaptive mechanisms to stressful environments. The results also showed CA expression rapidly increased to its highest level exposed to blue light, followed by UV light conditions. And under dark conditions, changes in CA expression were minimal. *C. sp. ICE-L* is stimulated to produce CA in response to sudden changes in the external environment; however, expression subsequently decreases, most likely because extended light exposure severely damages the algal cells, and expression is inhibited.

CAs have become drug development targets for numerous diseases [40–42]. This is one of the reasons that prompt us to consider CAs as a research subject. After anticipating and analysing, we now know that CA2 is

located in the chloroplast, which has important ecological significance for studying chloroplast α -CA. Another reason for our interest is that *C. sp. ICE-L* is widespread in the oceans and can effectively participate in the fixation of atmospheric CO₂ [6, 42].

Conclusion

In this study, the transcription of three different CAs was examined by qRT-PCR after exposure to UV light, blue light, and darkness. This experiment contributes to the exploration of the factors influencing CA expression in polar algae. In order to further study CAs, CA expression should be induced specifically under several other external conditions; this will be of great significance for the future study of *Chlamydomonas*.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval This article does not contain studies with animals.

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