Sequence analysis and function identification of α-type carbonic anhydrase (CA) in the gametophytes of *Saccharina japonica*

邮箱：M1242948000@163.com

Jiajian Weng[a],Yanhui Bi[b]

[a] Key Laboratory of Exploration and Utilization of Aquatic Genetic Resources Conferred by Ministry of Education, Shanghai Ocean University, Shanghai 201306, China

[b] International Research Center for Marine Biosciences Conferred by Ministry of Science and Technology, Shanghai Ocean University, Shanghai 201306, China

Abstract

The increase of CO_2 content in the atmosphere caused by human activities is the main reason for the intensification of greenhouse effect and ultimately global warming. As the main receiving reservoir of CO_2 emissions on the earth, the ocean has absorbed about 30% of the global CO_2 in the past 200 years, resulting in seawater acidification. Although the solubility of CO_2 in seawater is very low and the diffusion rate is very slow, the photosynthetic efficiency of kelp and other macroalgae is much higher than that of terrestrial plants, mainly because they have an inorganic carbon concentrating mechanism in order to increase the concentration of CO_2 around RubisCO site, thus improving the photosynthetic efficiency of algae. Carbonic anhydrase (CA) is a zinc containing metalloenzyme, which can transform CO_2 and HCO^{3-} into each other and help CO_2 to be fixed by photosynthesis. In this paper, we will provide new ideas for the research by synthesizing the current research..

Background

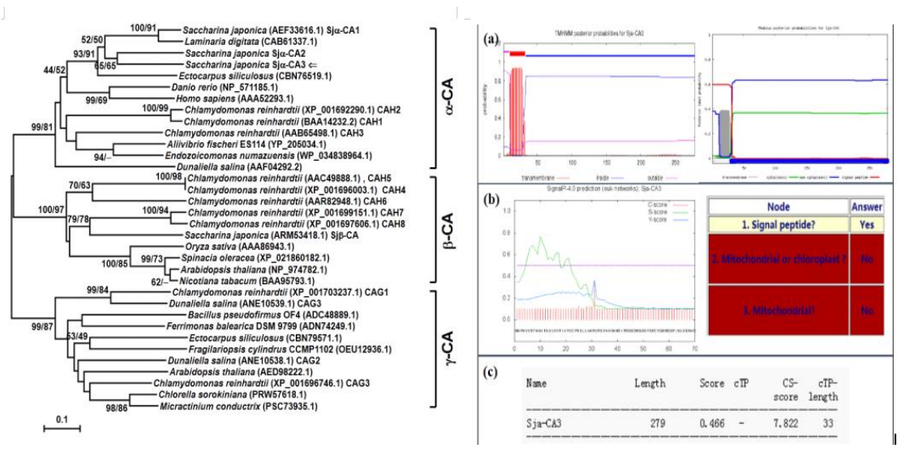
Under the condition that the sea floor is not as light as the land, kelp has the same or higher productivity as the most productive land plants (such as sugar cane). This shows that there is an inorganic carbon concentration mechanism in kelp and other large algae, which can efficiently use dissolved inorganic carbon (DIC) in water. It is known that DIC exists in seawater in the forms of CO_2, HCO^{3-}, CO and H_2CO^{3-} CO_2 can enter cells or organelles directly through biological membranes, but the solubility of CO_2 in seawater is low (1%) and the molecular diffusion speed is slow.

Most inorganic carbon exists in the form of HCO^{3-} (91%). HCO^{3-} needs to be converted into CO_2 through carbonic anhydrase inside and outside the cells, It is convenient for the utilization of ribulose 1,5-diphosphate carboxylase/oxygenase (RubisCO) in algae cells to improve the photosynthetic performance. At the same time, it can also convert CO_2 into HCO^{3-}, forming a Ci pool to store inorganic carbon. Therefore, carbonic anhydrase plays an important role in regulating the photosynthesis of algae

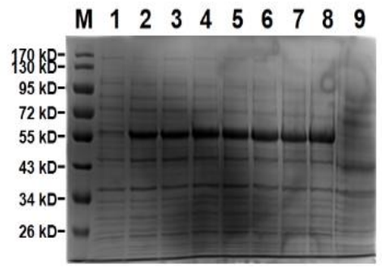
Materials and methods

1. Bioinformatics analysis of *Sjα*CA3
2. Prokaryotic expression of *Sjα*CA3
3. Enzyme activity determination of *Sjα*CA3

Results



TMHMM and Phobius software were used to conduct transmembrane analysis on *Sjα*CA3. The results showed that the amino acid at 10 His-32 Val starting from the N-terminal of the protein had a strong hydrophobic transmembrane region. The prediction result of SignalP shows that there is no signal peptide in *Sjα*CA3, but the prediction result of iPSORT shows that there may be a signal peptide with a length of 27 amino acids at the N end . ChloroP predicted that it had chloroplast transport peptide composed of 33 amino acids. After removing this region, a mature protein composed of 246 amino acids was obtained, with a relative molecular weight of 27.52 kD and an isoelectric point of 4.61.



M: Prestained Protein Ladder; Lanes 1 to 8: the whole-cell protein of *E. coli* BL21 after 0 h, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h and 7 h inducing with IPTG, respectively; Lanes 9 and 11: the whole-cell protein of *E. coli* BL21; Lane 10: the whole-cell protein of *E. coli* BL21 after 4 h inducing with IPTG.

Evaluation of CO_2 hydratase activity of Sjα-CA3

Name	Total vitality/U	Quality/mg	Specific activity(U/mg)	Average specific activity (U/mg)
Enzymatic group1	0.44	0.502	0.88	0.82±0.087
Enzymatic group2	0.43		0.86	
Enzymatic group3	0.36		0.72	

Evaluation of esterase activity of Sjα-CA3

Name	Total vitality/U	Specific activity（U）	Quality/g	Specific activity (U/g)	Average specific activity (U/g)
Enzymatic group1	0.001341	0.001344	0.000623	2.152	2.157±0.007
Enzymatic group2	0.001342			2.154	
Enzymatic group3	0.001348			2.164	

1.The specific activity of the hydration reaction of SjCA3 is 0.820.087 U/mg protein.

The specific activity of the enzyme is **2.**1570.007 U/g protein . The recombinant *Sj*αCA3, like other CAs, has not only hydratase activity, but also esterase activity.

Conclusions

1. The 10 His~32 Val amino acids starting from the N-terminal have a transmembrane region, a signal peptide with a length of 27 amino acids and a chloroplast transport peptide with a length of 33 amino acids. After removing 33 amino acids from the N-terminal, the molecular weight is about 27.52 kD, which may be a protein existing in the secretion pathway.

2. *Sjα*CA3 have the function of hydrating CO_2 to HCO^{3-} and hydrolyzing p-nitrophenyl acetate to p-nitrophenol, which proved that the gene belonged to the CA gene family.