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ORIGINAL ARTICLE

Computational analysis of physico-chemical properties and homology modeling of carbonic anhydrase from *Cordyceps militaris*

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Abstract In the present study, the protein sequence of carbonic anhydrase enzyme from *Cordyceps militaris* (accession no. EGX89555.1) was retrieved from GenPept database and subjected to computation for various physico-chemical properties, transmembrane segment prediction, homology modeling, domain identification and structural alignment. Five potential transmembrane segments of various lengths were predicted by transmembrane prediction server. The 3D structure of this protein was determined by homology modeling using 3d jigsaw server. A single eukaryotic-type carbonic anhydrase domain belonging to Carb_anhydrases family domain was identified at two positions from 45 to 153 and 232 to 302 residues region. This modeled structure showed 100 % structural similarity with human carbonic anhydrase III (PDB ID: 1z93-A) in PDB database.

Keywords Carbonic anhydrase · *Cordyceps militaris* · Physico-chemical properties · Homology modeling · Domain

1 Introduction

Carbonic anhydrase (CA) enzyme was first reported in vertebrate erythrocytes (Brinkman et al. 1932). It forms a family of enzymes that catalyze the rapid interconversion of carbon dioxide and water to bicarbonate and protons (or vice versa), a reversible reaction that occurs rather slowly in the absence of a catalyst (Badger and Price 1994). This enzyme plays a central role in facilitating the diffusion of carbon dioxide in photosynthesis, but it is also essential in areas such as ion balance, respiration and an important role in biosynthesis or detoxification pathways that use HCO_3 as a co-factor or as a co-substrate, such as fatty acid or arginine biosynthesis, the cAMP pathway and cyanate degradation (Aguilera et al. 2005; Anderson et al. 1990; Bahn and Mühlschlegel 2006). The *Cordyceps militaris* occurs throughout much of the Northern Hemisphere as a pathogen of lepidopteran insect pupae (Sung et al. 2007). *C. militaris* is readily characterized by the sexual fruiting bodies forming on mycosed pupae, the structures giving the fungus its common name of 'pupa grass' in China. The *C. militaris* is best known as traditional Chinese medicines (Paterson 2008). Extracts from both mycelium and fruiting bodies of *C. sinensis*, *C. militaris* and other *Cordyceps* species showed significant anticancer activities by various mechanisms such as, modulating immune system and inducing cell apoptosis (Khan et al. 2010). Over expression of carbonic anhydrase III reduces steady-state levels of intracellular reactive oxygen species, increases proliferation rate and protects cells from hydrogen peroxide-induced apoptosis (Raisanen et al. 1999). In light of above the homology modeling of carbonic anhydrase in *C. militaris* and its analogy with carbonic anhydrase report in human system becomes very important in understanding the much acclaimed role of *C. militaris* as an antiaging, anticancerous and immunomodulating agent. Considering the above facts, the study of amino acid sequence of carbonic anhydrase from *C. militaris* is a very interesting task. In this communication, we performed the *in-silco* analysis of *C. militaris* carbonic anhydrase to find out the physical and chemical properties like molecular weight, theoretical pI, atomic composition, estimated half-life, instability index, aliphatic index and grand average of hydropathicity

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on the basis of their amino acid sequence. Transmembrane segment prediction, domain identification and the automatic comparative modeling of carbonic anhydrase from *C. militaris* were also performed.

2 Materials and methods

The protein sequence of carbonic anhydrase enzyme from *C. militaris* (accession no. EGX89555.1) was searched and retrieved from GenPept database available at NCBI website (http://www.ncbi.nlm.nih.gov/protein). Computation of various physical and chemical properties including the molecular weight, theoretical pI, atomic composition, estimated half-life, instability index, aliphatic index and grand average of hydropathicity was calculated using ProtParam tool (http://web.expasy.org/protparam/) (Gasteiger et al. 2005). Transmembrane segment prediction was performed using "DAS"-Transmembrane Prediction server (http://www.sbc.su.se/~miklos/DAS/) (Cserzo et al. 1997). Homology modeling was performed using 3D jigsaw server (http://bmm.cancerresearchuk.org/~3djigsaw/) (Bates et al. 2001) and modeled structure was visualized and annotated under Rasmol program and SAS (http://www.ebi.ac.uk/thornton-srv/databases/sas/). Domain was identified using Pfam database search program (http://pfam.

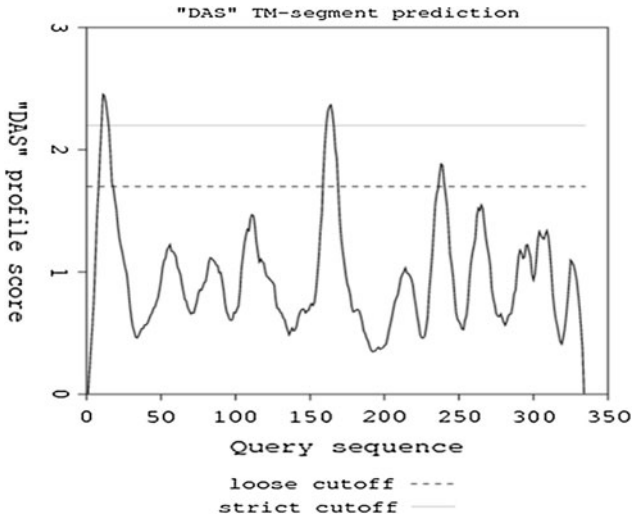

Fig. 1 Transmembrane segment prediction by DAS server

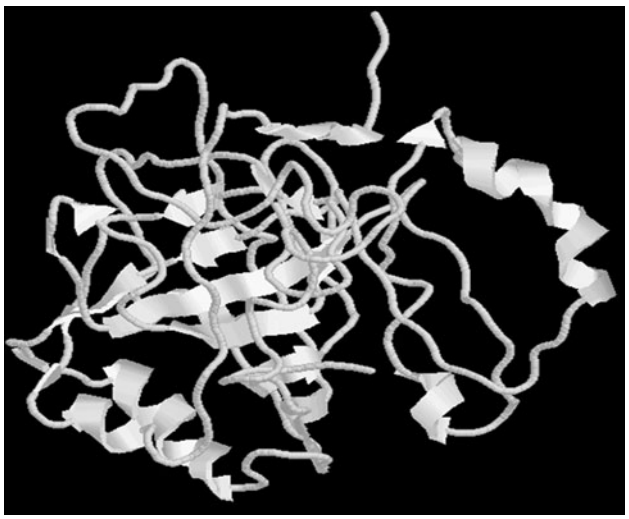

Fig. 2 3D structure of carbonic anhydrase from *Cordyceps militaris* fungus modeled by 3d jigsaw server and visualized under Rasmol program

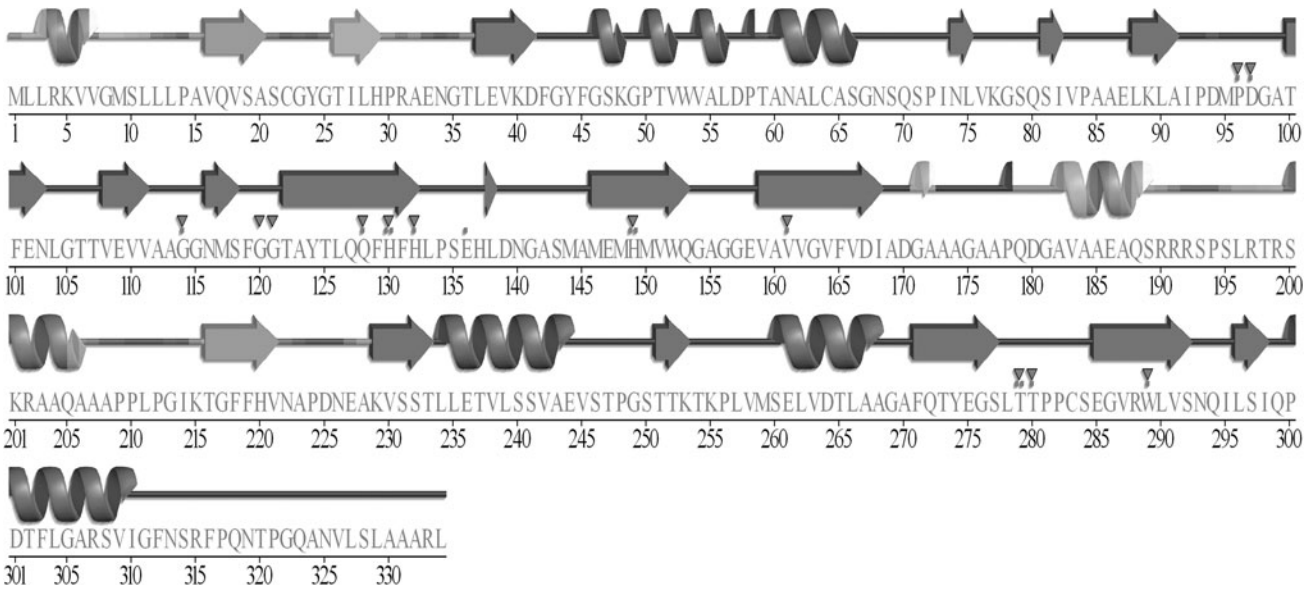

Fig. 3 Model analyzed under SAS server available at EBI

No:	Chain	Z	rmsd	lali	nres	%id	PDB	Description
1:	1z93-A	99.9	0.5	263	263	100	PDB	MOLECULE: CARBONIC ANHYDRASE III;
2:	1z97-A	45.5	0.0	263	263	100	PDB	MOLECULE: CARBONIC ANHYDRASE III;
3:	1flj-A	44.5	0.6	257	259	91	PDB	MOLECULE: CARBONIC ANHYDRASE III;
4:	2hfy-A	44.3	0.5	257	259	99	PDB	MOLECULE: CARBONIC ANHYDRASE 3;
5:	2hfw-A	44.2	0.5	257	257	99	PDB	MOLECULE: CARBONIC ANHYDRASE 3;
6:	2hfx-A	44.1	0.6	257	259	99	PDB	MOLECULE: CARBONIC ANHYDRASE 3;
7:	3daz-A	41.7	1.0	257	257	59	PDB	MOLECULE: CARBONIC ANHYDRASE 2;
8:	3dc9-A	41.7	1.0	257	257	59	PDB	MOLECULE: CARBONIC ANHYDRASE 2;
9:	1th9-A	41.7	1.0	257	258	59	PDB	MOLECULE: CARBONIC ANHYDRASE II;
10:	1thk-A	41.7	1.0	257	258	59	PDB	MOLECULE: CARBONIC ANHYDRASE II;
11:	1i9m-A	41.7	1.0	257	258	59	PDB	MOLECULE: CARBONIC ANHYDRASE II;
12:	3dbu-A	41.7	1.0	257	257	59	PDB	MOLECULE: CARBONIC ANHYDRASE 2;
13:	2nwy-A	41.7	1.0	257	258	59	PDB	MOLECULE: CARBONIC ANHYDRASE 2;
14:	2h4n-A	41.7	1.0	257	257	59	PDB	MOLECULE: CARBONIC ANHYDRASE II;
15:	2osf-A	41.6	1.0	257	257	59	PDB	MOLECULE: CARBONIC ANHYDRASE 2;
16:	2abe-A	41.6	1.0	257	257	59	PDB	MOLECULE: CARBONIC ANHYDRASE II;
17:	1zsb-A	41.6	0.9	257	257	59	PDB	MOLECULE: CARBONIC ANHYDRASE II;
18:	1rzc-A	41.6	1.0	257	258	59	PDB	MOLECULE: CARBONIC ANHYDRASE II;
19:	1h4n-A	41.6	1.0	257	257	59	PDB	MOLECULE: CARBONIC ANHYDRASE II;
20:	1g52-A	41.6	1.0	257	258	59	PDB	MOLECULE: CARBONIC ANHYDRASE II;
21:	1g46-A	41.6	0.9	257	258	59	PDB	MOLECULE: CARBONIC ANHYDRASE II;
22:	1teq-X	41.6	1.0	257	258	59	PDB	MOLECULE: CARBONIC ANHYDRASE II;
23:	1cni-A	41.6	1.0	257	257	59	PDB	MOLECULE: CARBONIC ANHYDRASE II;
24:	1yo2-A	41.6	1.0	257	258	59	PDB	MOLECULE: CARBONIC ANHYDRASE II;
25:	1yo1-A	41.6	1.0	257	258	59	PDB	MOLECULE: CARBONIC ANHYDRASE II;
26:	2nxr-A	41.6	1.0	257	258	59	PDB	MOLECULE: CARBONIC ANHYDRASE 2;
27:	1cnh-A	41.6	1.0	257	257	59	PDB	MOLECULE: CARBONIC ANHYDRASE II;
28:	1i9q-A	41.6	1.0	257	258	59	PDB	MOLECULE: CARBONIC ANHYDRASE II;
29:	1g3z-A	41.6	1.0	257	258	59	PDB	MOLECULE: CARBONIC ANHYDRASE II;

Fig. 4 Structure of *C. militaris* carbonic anhydrase showing 100 % similarity with human carbonic anhydrase III (PDB ID: 1z93-A) in PDB database

sanger.ac.uk/search) (Finn et al. 2010). Structural alignment was performed using Dali server (http://ekhidna.biocenter.helsinki.fi/dali_server/start).

3 Results and discussion

The amino acid sequence of carbonic anhydrase enzyme from *C. militaris* fungus was searched and retrieved from GenPept database of NCBI along with the accession number XP_001389200.2. The computation of various physical and chemical properties result revealed that atomic composition consisted of 1,527 carbon, 2,428 hydrogen, 422 nitrogen, 471 oxygen and 12 sulfur atoms and molecular weight was found to be 34.6 kD. The theoretical pI was estimated to be 5.87. Half-life, instability index, aliphatic index and grand average of hydropathicity were 20 h, 48.6, 85.93 and 0.092, respectively. Instability index was >40 so this protein is unstable. Five potential transmembrane segments of various lengths were predicted by transmembrane prediction server (Fig. 1). First segment was nine residues long and found from 9 to 17 residues region with 1.7 cutoff value, second segment was four residues long and found from 11 to 14 residues region with 2.2 cutoff value, third segment was ten residues long found from 159 to 168 residues region with 1.7 cutoff value, fourth segment was five residues long found from 161 to 165 residues region with 2.2 cutoff value and fifth segment was four residues long found from 237 to 240 residues region with 1.7 cutoff value. Homology modeling of retrieved amino acid sequence was performed using 3d jigsaw server to get the 3D coordinates. The model structure was visualized and annotated using Rasmol program and SAS server respectively (Figs. 2, 3). Using Rasmol program it was found that the 3D structure of carbonic anhydrase enzyme from *C. militaris* consisted of 9 alpha helices, 18 beta strands and 34 turns, stabilized by 123 hydrogen bonds. The domain identification result showed single eukaryotic-type carbonic anhydrase domain belonging to Carb_anhydrases family domain at two positions from 45 to 153 and 232 to 302 residues regions. Structural alignment of *C. militaris* carbonic anhydrase using Dali server showed 100 % similarity with human carbonic anhydrase III (PDB ID: 1z93-A) in PDB database (Fig. 4).

4 Conclusions

The amino acid sequence-based physico-chemical properties analysis of *C. militaris* carbonic anhydrase revealed that this protein is unstable with 34.6 kD molecular weight and carries no net electrical charge at 5.87 pH value. Homology modeling result showed that structure of this protein consisted of 9 alpha helices, 18 beta strands and 34 turns which were stabilized by 123 hydrogen bonds and single eukaryotic-type carbonic anhydrase domain belonging to Carb_anhydrases family identified at two positions (45–153 and 232–302) using Pfam database. Modeled structure showed 100 % similarity with human carbonic anhydrase III (PDB ID: 1z93-A) in PDB database. The structural similarity of carbonic anhydrase of *C. militaris* with Carbonic anhydrase III of human provides evidence for its role as an antiaging, anticancerous and immunomodulating agent. However, owing to the considerable importance of carbonic anhydrase in *C. militaris*, more contributions are warranted for the detailed investigation of the functional properties of this enzyme with respect to human carbonic anhydrase III.

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