Carbonic Anhydrase II Based Biosensing of Carbon Dioxide at High Temperature: An Analytical and MD Simulation Study

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on the basis of the outcome obtained from MD simulations, in which behavior was analyzed at three different temperatures for 50 ns time scale. To validate the outcome of MD simulations, CAII was sub-cloned cDNA of human CAII in the expression vector pET15d. Open reading frame (ORF) for CAII is of 801 base pairs encoded 267-amino acids. We constructed vector including the sequence coding for a CAII protein and an N-terminal poly-histidine (6XHis) tag to simplify the detection of protein expression using antibodies specific for the tag. Purification of recombinant protein was performed using immobilized metal affinity chromatography followed by gel filtration. The purified protein was used for precipitation of CO_2 (aq) into $CaCO_3$ in the presence of calcium ions at high temperature (325 K). Furthermore, the CAII based detection of the CO_2 at 325 K was analyzed by using the electrochemical methods.

Materials and Methods

Materials

E. coli strains DH5α (Invitrogen, California, USA) and Origami BL21 (DE3) (Novagen, Wisconsin, USA) were used for cloning and expression of recombinant protein, respectively. The *E. coli* cells harboring recombinant plasmids were grown aerobically in Luria-Bertani (Merck, Darmstadt, Germany) broth with 100 µg/µl Ampicillin (Sigma, Saint Louis, MO, USA). Plasmid pET15d (Novagen, Wisconsin, USA) was used as an expression vector. Plasmid isolation, restriction enzyme digestion, ligation, and competent cell preparation were carried out by standard procedures.

Molecular dynamic simulations

The structure of CAII was modelled by using the MODELLER module of the Discovery Studio 2016 (DS, http://accelrys.com/products/collaborative-science/biovia-discovery-studio/). The modelled structure of CAII was minimized by using the CHARMM force field [20] based optimization modules of DS. Similarly, the structure of CO_2 was constructed by utilizing the drawing utilities present in the DS and structure was optimized by using the DFT method implemented in the Dmol3 module of the DS.

Furthermore, the molecular docking was performed by using the CDOCKER module which is a CHARMM [20] force field based docking algorithm implemented in DS. The understanding about the active site of the CAII was obtained from the literature [21]. The CO_2 was docked in the active site of CAII and around 10 conformations generated for the further study. The generated conformations were re-scored, and best docked pose was selected for the MD simulation studies.

The selected docked structures of CAII and CO_2 were subjected to MD simulations using GROMACS [22] (version 5.1.2, installed on the Center for High Performance Computing (CHPC), Cape Town which provide 10 nodes with 24 cores per node of space for computation). The topology of CAII was produced on the basis of GROMOS96 53a6 force field [23]. Due to the unavailability of suitable force field parameters for drug-like molecules in the GROMACS package, the PRODRG server [24] was used for the generation of the CO_2 topologies and coordinate files. The partial charges were corrected by using DFT method of Gaussian which utilized the B3LYP 6-31G (d,p) basis set and CHELPG program [25]. After the successful topology generation, the docked complex was immersed in SPC/E water model [26] and the system was neutralized by adding the counter ions. The neutralized system was energetically minimized by utilizing the steepest descent and conjugate gradient algorithms with a convergence criterion of 0.005 kcal mol^{-1}. In order to increase the reliability of the MD simulations the restrains were applied to the structure of the piperine before the equilibration phase.

The equilibration phase was carried out separately in NVT (constant volume) as well as NPT (constant pressure) ensemble conditions, each for 100 ps time scale. The temperature of the system was changed from 300 K-325 K in both ensemble conditions along with pressure which was maintained at 1 bar by utilizing Parrinello-Rahman barostat in constant pressure ensemble. The

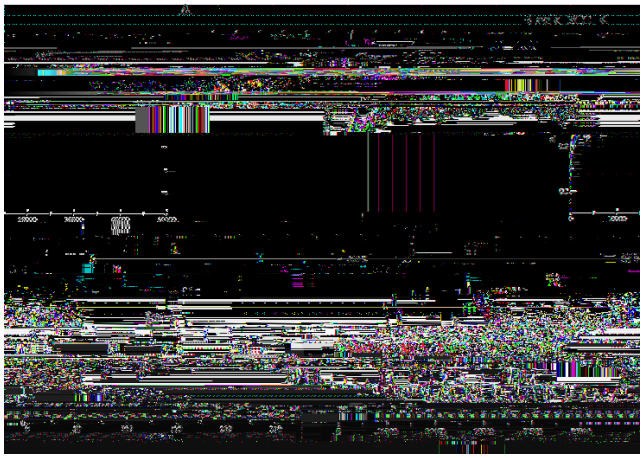

Figure 2: The graphical representation of the variations in (A) RMSD values at different temperature (B) Rg values at diverse temperature (C) RMSF values.

T erefore, measuring the pH is a viable method to monitor the progress of this enzymatic reaction at high temperature 325 K. Figure 4 shows the decrease in pH during the assay for CA using the enzyme obtained. T e curves show that this enzyme was a very e fective catalyst for hydration of CO_2.

Acceleration of carbonate formation by CA

In all studied conditions the distance between the two entities of the complex were remain relatively similar (Figure 1), which was observed in the range of 0.2 nm-0.3 nm. Furthermore, the RMSD values of the Cα atoms showed a varied nature. At 300K, the RMSD values were fuctuating around 0.3 nm up to 40 ns and the afer that the magnitude

hydration activity and converts CO_2 to $CaCO_3$ very e f ciently. T e potential for acceleration of CO_2 capture impressively illustrate the importance of CAII. T e present work showed the biomineralization process for CO_2 hydration into calcium carbonate at high temperature. Furthermore, the voltammetric studies con f rmed that CAII can successfully detect CO_2 in solution through the electrocatalytic performance of glassy carbon electrode even at 325 K. T ese results are very encouraging for the future development in which the electrode can be turned into an ideal tool of a CAII based biosensor. Moreover, the MD simulations results have been validated by *In vitro* studies that the CAII can detect the CO_2 even at higher temperatures and can be used for the detection of the pollutant CO_2. T e studies showed the stability of CAII under high temperature (325 K) and con f rmed the poten

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