ROLE OF THE CHARGES OF LYSINE SIDE CHAINS IN THE INTERACTION OF BOVINE CARBONIC ANHYDRASE WITH SODIUM DODECYL SULFATE: MOLECULAR DYNAMICS SIMULATION APPROACH

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In this work, the molecular dynamics (MD) method was used for the study of the interaction of bovine carbonic anhydrase with sodium dodecyl sulfate (SDS), with emphasis on the role of the charges of lysine side chains. For this purpose, bovine carbonic anhydrase was considered in two forms, namely BCA (with eighteen lysine-NH_2 groups) and BCA-H (with eighteen lysine- NH_3^+ groups). Simulation of each of these proteins was performed in two different concentrations of SDS. The following parameters: root mean square deviation of C-alpha of backbone, radius of gyration, hydrophobic solvent accessible surface area and the number of protein hydrogen bonds were calculated for BCA and BCA-H in the two concentrations of SDS. With regard to these parameters, no considerable differences were observed between BCA and BCA-H. In spite of the noted similar behavior, it was specified that these two structures were different concerning residues flexibility. Also, the number of SDS molecules bonded to these two structures was different.

Key words: bovine carbonic anhydrase, sodium dodecyl sulfate, MD simulation.

INTRODUCTION

A review of effective factors involved in the stability of proteins is important in connection with applicability. Although it is believed that hydrophobic and hydrogen bonding interactions are mainly responsible for the stability of proteins (1), the involvement of electrostatic interactions in protein stability is not yet openly clear and varies from one protein to another (2–4).

One of the methods for changing the charge of a protein is the chemical method. Different research groups have used this method to study the effect of

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electrostatic interactions on protein stability (5–8). Recently, Withesides *et al*. have used this method for studying the role of the charges of lysine side chains on the stability of BCA (8). They investigated denaturation of BCA and peracetylated BCA (BCA-Ac18) induced by sodium dodecyl sulfate (SDS) and concluded that large changes in charge do not play a considerable role in the structure of this enzyme in the presence of SDS (9–13). The question raised is that if changes in charge outside of active site of BCA do not have any effect on the secondary and tertiary structures, then what is the role of charged lysine residues on its surface?

In this study, the molecular dynamics (MD) method was used to study the interaction of BCA and SDS, and efforts were made to review the effect of charges of the lysine side chain on protein in detail.

MATERIAL AND METHODS

All calculations were carried out using Gromacs3.3.1 package (14) and Gromos96 force field (15). The components of the simulation boxes for the given systems were as follows: dimension 7×7×7, the number of water molecules was 10000 and the number of ligand molecules was 4 or 8. It should be noted that the number of water molecules and ligands was taken from the reference (8). The span time of the simulation was 5 ns. A steepest-descent algorithm was performed to minimize the energy of each system and to relax the water molecules. The MD simulation for each system was carried out in two stages. In the first stage, position-restrain simulation was conducted, in which the atoms of the protein molecule were held fixed, whereas the water molecules were free to move around so that they would reach the equilibrium state. In the second stage, each system was simulated with a time step of 0.2 fs. LINCS algorithm (16) was employed to fix the chemical bonds between the atoms of the protein and SETTLE algorithm (17) in the case of water molecules. The atoms in the system were given initial velocities according to Maxwell-Boltzmann distribution at 300°C. To maintain a constant temperature and pressure for various components during simulations, Brendsen coupling algorithm (16, 18) for each component of the system, with the relaxation times of 0.1 and 0.5 ps, respectively, was used. As the systems involve many positive and negative charges, PME algorithm was applied to estimate the electrostatic interactions. In this algorithm every atom interacts with all atoms in the simulation box and all of their images constitute an infinite number of identical copies surrounding the main box, so that satisfactory results are produced from the electrostatic interactions (19).

RESULTS AND DISCUSSION

In order to study the structural behavior of BCA and BCA-H when interacting with SDS, four parameters, *i.e.* root mean square deviation of C-alpha

of backbone ($C_\alpha - RMSD$), radius of gyration (R_g), hydrophobic solvent accessible surface area (HSAS) and number of protein hydrogen bonds (HB), were calculated for these two forms, for two different concentrations of SDS.

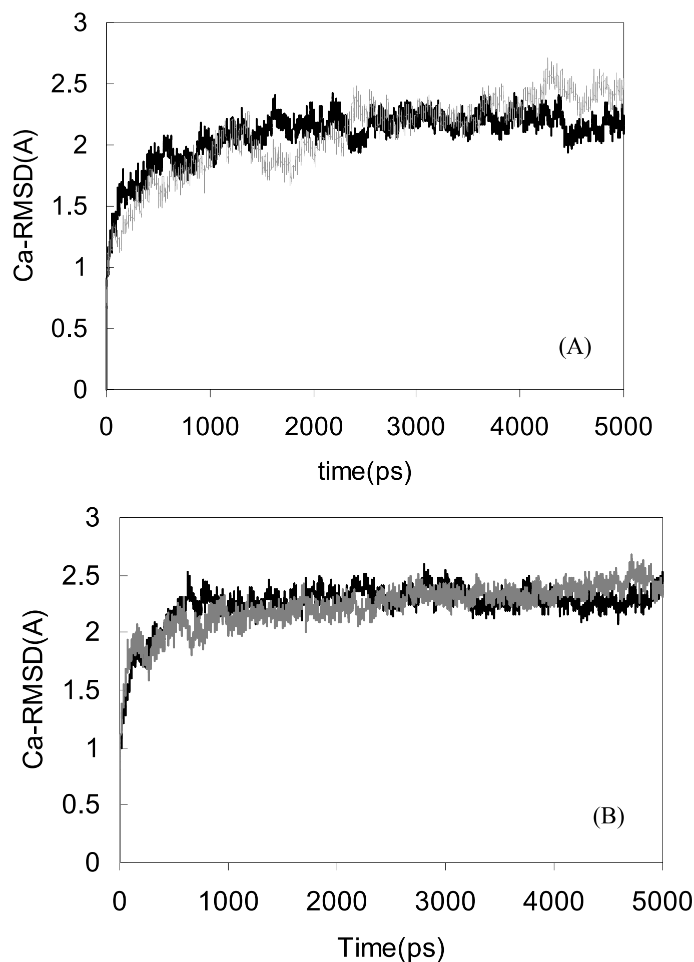

Fig. 1. – Time evolution of the $C_\alpha - RMSD$ of BCA and BCA-H for SDS=4 (A) and SDS=8 (B).

In Figures 1 and 2, changes in $C_\alpha - RMSD$ for BCA and BCA-H in the presence of 4 and 8 SDS molecules are shown. Quantities of $C_\alpha - RMSD$ were calculated in connection with their primary configuration. Regarding these figures, the following results have been obtained:

1) Quantities of $C_\alpha - RMSD$ for BCA and BCA-H change maximum up to 2.5 Å. Trivial fluctuation for periods longer than 1ns shows stability of protein structures towards SDS. BCA has a unique knot structure in the C-terminal region

and has ten β-strands and seven α-helices that cause resistance of protein against SDS (20, 21).

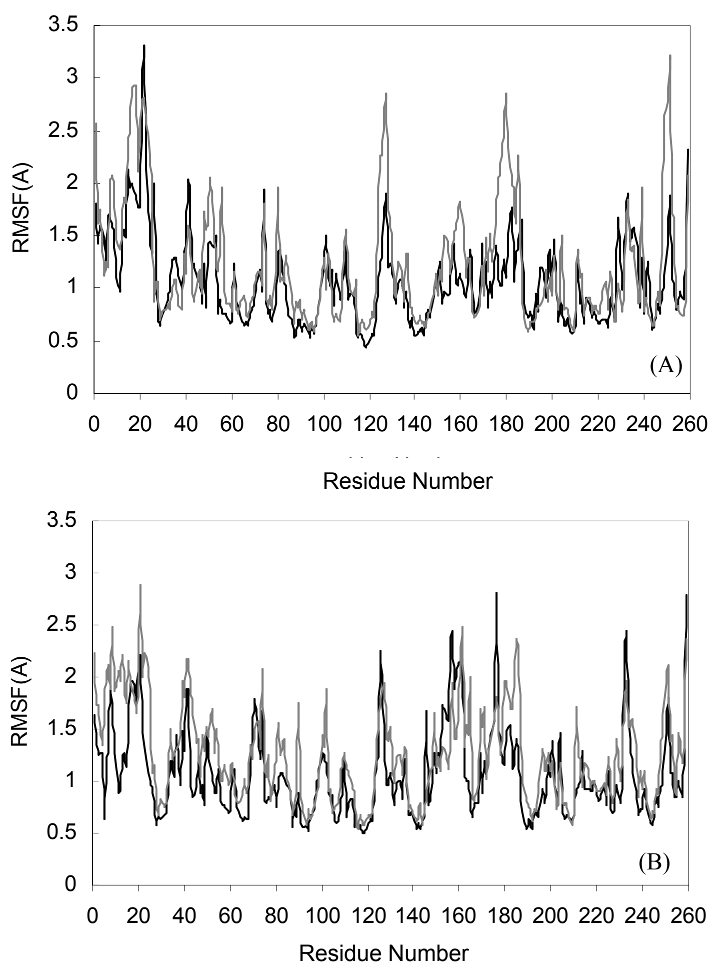

Fig. 2. – RMSF *vs* residue number for the BCA and BCA-H: (A) SDS=4 and (B) SDS=8.

2) Changes in quantities of $C_α$–*RMSD* for BCA and BCA-H during simulation were less than 0.5 Å, which shows that changes in charge from –1 to –19 (for BCA-H and BCA, respectively) do not have a considerable effect on the structure of the enzyme. Also, changes in R_g, HSAS and HB for the enzyme in two forms with different charges show that there is no considerable difference between the behavior of BCA and BCA-H when interacting with SDS (support information). The above noted results are consistent with the experimental results of Withesides *et al.* (8).

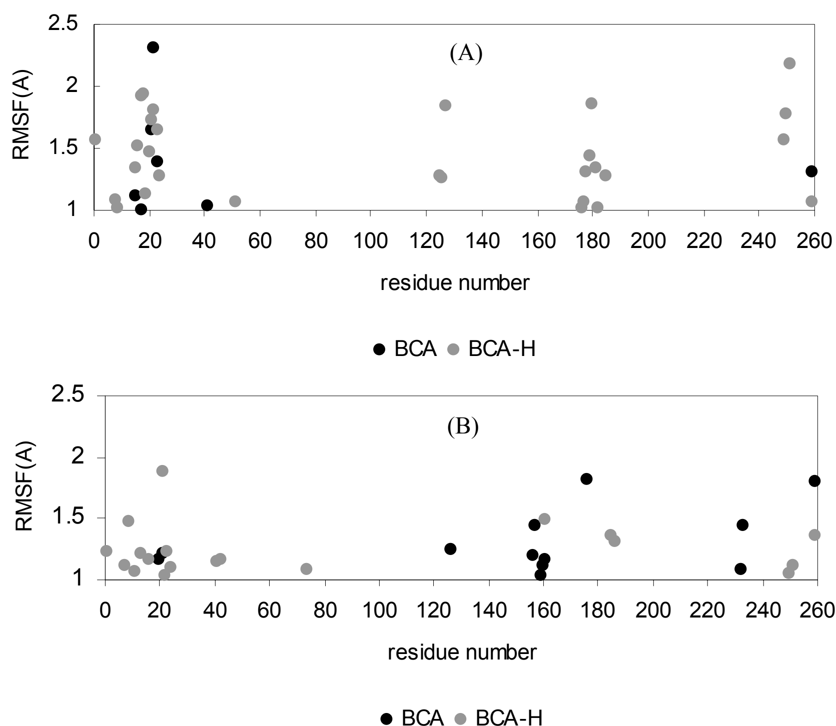

Fig. 3. – Residues with RMSF greater than 1Å: (A) SDS=4 and (B) SDS=8.

To make a more detailed study of the interaction of BCA and BCA-H with SDS, changes in residue mean square fluctuation (RMSF) were calculated and the numbers of bonded SDS molecules were evaluated in each case. Changes in RMSF in the presence of 4 and 8 molecules of SDS are shown in Figures 3 and 4. In order to make a better analysis and draw conclusions, residues with RMSF greater than 1 Å were selected from Figures 3 and 4 and shown in Figures 5 and 6. If we consider these residues with more flexibility, the patterns of obtained structures for BCA and BCA-H will be different, which means that the positions of the flexible residues are considerably different in the two structures. Since the charges for BCA and BCA-H are different and knowing that SDS has hydrophobic tail and hydrophilic head, it is expected that its interaction with these two structures will be different. Also, the effect of different concentrations of SDS on these two structures appears as the difference in the number of flexible residues and is observable in the figures.

To determine the number of bonded SDS, the distance between SDS molecules and each of the BCA-H and BCA structures within the range of less than 4 Å were calculated, because this range was accepted as electrostatic interactions

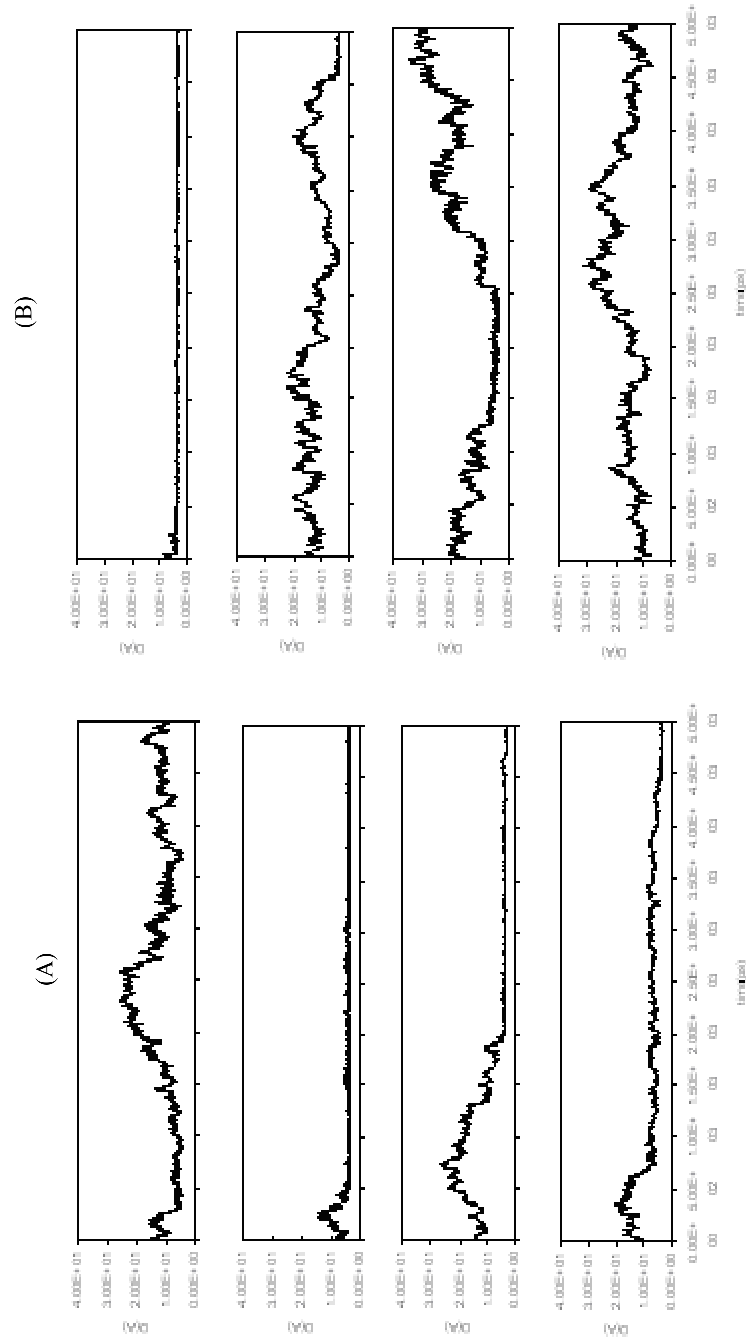

Fig. 4. – The minimum distances between the SDS atoms and the BCA atoms (A) or BCA-H atoms (B), for SDS=4.

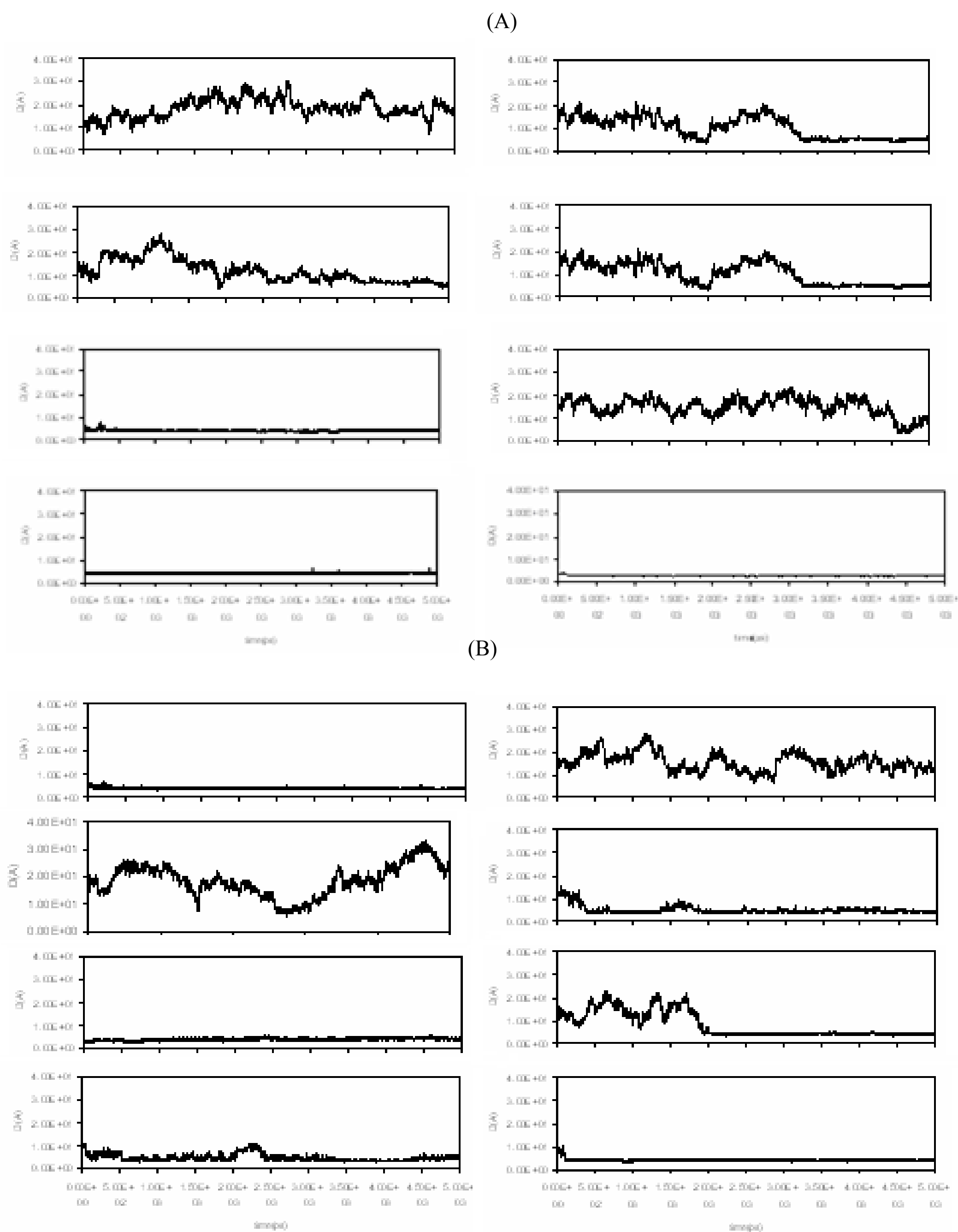

Fig. 5. – The minimum distances between the SDS atoms and the BCA atoms (A) or BCA-H atoms (B), for SDS=8.

(8, 22, 23). Figures 7 to 10 show the result of this calculation. The number of SDS bonded molecules is obtained and reported in Table 1. One can observe that the numbers of SDS molecules bonded to each protein structure have increased with the increase in SDS concentration. The results are due to the dual nature of SDS.

Table 1

The number of SDS molecules bounded to BCA and BCA-H

No. of SDS molecules	**No. of bonded SDS molecules**	
	BCA	**BCA-H**
4	3	1
8	4	6

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