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A study on chirality in biomolecules: the effect of the exchange of L amino acids to D ones in Sso7d ribonuclease

Abstract In this work, the conformational behavior of ribonuclease Sso7d is studied as a function of chirality of its constituting amino acids. Both optimized structures (using molecular mechanics with the CHARMM force field) and dynamic behavior (obtained by molecular dynamic simulations) are compared.

Keywords Conformation of Sso7d ribonuclease · Chirality perturbation · Mutation · Homochirality

Introduction

As is well known for all living organisms on the Earth, proteins contain nearly exclusively L- and no D-amino acids [1]. In addition, in DNA there is only D- and no L-desoxyribose [2]. The origin of this phenomenon (homochirality or dissimilarity in Pasteur's original word) is constantly in the focus of the attention of the scientific community.

After the discovery of L-homochirality, it was recognized that there are still a few cases where D-amino acids occur in nature. First it was noticed that they are present in the cell wall of microorganisms [3]. Later D-aspartic acid was found in various aged human tissues (tooth, bone, brain etc.) and D-serine was detected in beta-amyloid protein of Alzheimer's disease [4]. The presence of D-Asp in aged tissues has been explained as a result of epimerization. Also, D-amino acids were found in smaller biologically important peptides (for instance, the opioid dermorphin contains D-Ala, whose

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substitution by L-Ala causes the peptide to lose its activity). D-Ala was recently used (through exchanging several glycine residues) to increase protein stability by relieving strain in the folded state of proteins NT9 and UBA [5]. These results suggest that D-amino acids are attracting more and more attention both in living systems and in protein engineering. This is why we aim to understand the conformational behavior of mutated (D-amino acid substituted) proteins in this paper.

The catalytic activity of enzymes depends strongly on their conformation, especially on the conformation of their active sites. This conformation is significantly influenced by the chirality of the residues occurring in enzymes, since if one simply alters the position of side chains in residues, then the resulting structure may even contain steric hindrances. In addition, L-amino acids are known to prefer right-handed helices, while D-amino acids would rather be present in left-handed helices. Thus, changing the chirality of some (or all) of the residues in a protein is expected to change not only the local structure of the molecule, but may also have an impact on the whole secondary (and higher order) structure.

To demonstrate the process of the change (generated by altering the chirality of certain residues) in the structure of enzymes, we have performed a chirality-perturbation study on ribonuclease Sso7d [6, 7] (a small, single domain, frequently occurring protein from the thermoacidophilic archaeon *Sulfolobus solfataricus*; endowed with DNA binding properties, RNase activity, and the capability of rescuing aggregated proteins in the presence of ATP). This model system has been chosen for this study because it consists of only 62 residues. Its active site was identified by Consonni et al. [7] and consists of four residues Tyr7, Lys12, Tyr33, Glu35.

The relative orientation of these residues and the overall change in the secondary structure of the enzyme was studied by geometry optimization using MM (molecular mechanics) in the presence of water. Conformational changes due to finite temperature were modeled by molecular dynamics (MD) simulation conducted at 310 K. This computation was performed on various forms of Sso7d: altering the chirality both in the residues of the active site and in different regions of the enzyme. One should point out that this is the first

example of demonstrating the conformational changes on a protein by changing the chirality at several residues or at larger sequences including the whole protein.

Materials and methods

The initial form of Sso7d was downloaded from the Protein Data Bank [8] (PDB entry 1JIC). It has the following primary structure (the members of the active site marked in bold face):

Ala Thr Val Lys Phe[5] Lys **Tyr**[7] Lys Gly Glu[10] Glu **Lys**[12] Gln Val Asp[15] *Ile* Ser Lys Ile Lys[20] Lys Val Trp Arg Val[25] Gly Lys Met *Ile* Ser[30] Phe *Thr* **Tyr**[33] Asp **Glu**[35] Gly Gly Gly Lys *Thr*[40] Gly Arg Gly Ala Val[45] Ser Glu Lys Asp Ala[50] Pro Lys Glu Leu Leu[55] Gln Met Leu Glu Lys[60] Gln Lys

Among these, Thr and Ile have two asymmetry centers (marked in italics). We have studied the following modified versions of the original enzyme (these notations are followed in the Figure captions later):

I. The chirality of a sequence of residues not containing the active site was altered.

    a. Residues 1–6 were transformed to their D isomers.
    b. Residues 13–32 were transformed to their D isomers.

II. The chirality of some (or all) of the residues belonging to the active site were altered.

    a. Tyr7 was transformed to its D form.
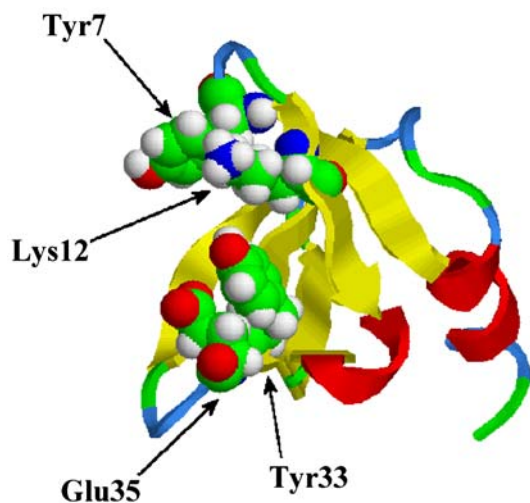   b. Lys12 was transformed to its D form.

Fig. 1 The optimized conformation of the original molecule with the indication of the residues which form the active site
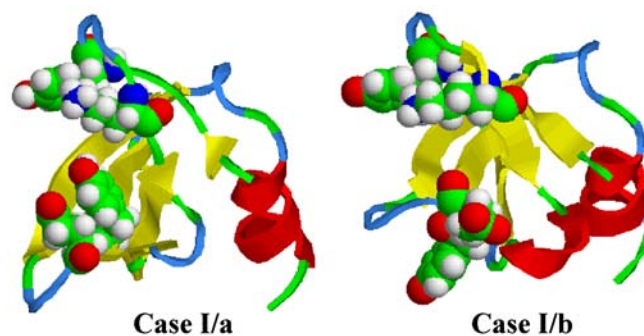

Fig. 2 Conformations gained by changing chirality in residues not part of the active site

    c. Tyr33 was transformed to its D form.
    d. Glu35 was transformed to its D form.
   e. All these four residues were transformed to their D forms.

III. All residues of Sso7d ribonuclease were transformed to their D forms.

These transformations were achieved by taking the mirror image of the side chain and the alpha hydrogen atom in the corresponding residues with respect to the plane spanned by the alpha carbon atom, the nitrogen atom and the carbon atom belonging to the carbonyl group (by using our simple software created to accomplish this goal). This way the second chiral centers in Ile and Thr residues remained untouched. (That is why Case III is not a mirror image of the original protein).

In some of the cases, the changes caused serious steric inhibitions, but even in those cases when these inhibitions were moderate, the structures were not necessarily close to their optimized forms. (Meaning the closest energy minimum on an obviously multiminima energy surface.) To resolve this problem, we optimized these structures by means of molecular mechanics using the CHARMM 22 force field [9] (developed for proteins). The presence of water was taken into account using a continuum solvent model developed by Still's group [10]. To be able to make a balanced comparison, the original (non-modified) structure was also optimized using the same method.

To study the changes of the relative position of the active site residues and the overall secondary structure of Sso7d ribonuclease at finite temperature, a molecular dynamics simulation was performed. A 100 ps long canonical (constant temperature and volume) simulation using 1 fs time steps was conducted using the Beeman integrator [11]. Both the MM optimization and the MD simulation were performed using the TINKER package [12].

Fig. 3 Conformations in the case of changing chirality in residues belonging to the active site
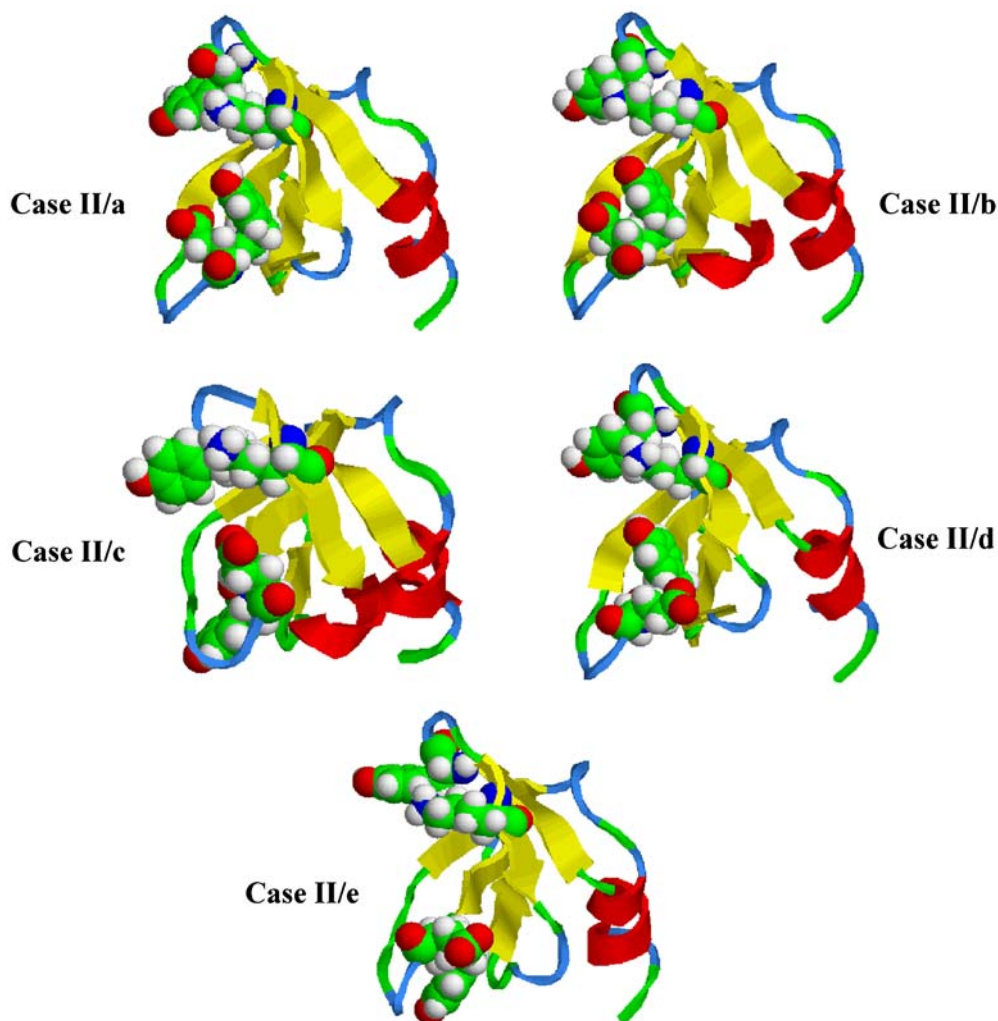
Results

The structures obtained by MM optimizations are displayed below[1]:

Figure 1 shows the optimized conformation of the original molecules (the residues which form the active site are indicated by arrows). As Case I/a (Fig. 2) shows, changing the chirality at the beginning of the protein chain, the active site remains almost unchanged, but the secondary structure of some of the central part of the enzyme loses its definite sheet character, corresponding to a similar change at the beginning of the protein chain. A more radical effect is made by changing the chirality in residues 13–32 (Case I/b, Fig. 2), which alters the shape of the active site. The chirality exchange in the residues of the active site (Cases II/a–II/e, Fig. 3), significantly alters the relative position of the side chains of the active-site residues. However, the secondary structure is still reminiscent of that of the original conformer. Exchanging all the L-residues to their D-counterparts (Case III on Fig. 4) has a major effect not only on the form of the active site, but also on the overall structure of the protein.
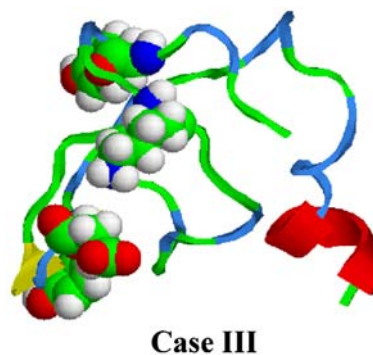

Fig. 4 Conformation gained by changing chirality in all the residues of the enzyme

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[1] The pictures were created by RasMol. [13] Active-site residues are displayed with van der Waals spheres while a ribbon representation is used for the rest of the molecule. Red ribbons denote helices, yellows are for sheets and turns are blue. Parts without defined secondary structure are green.

Table 1 The size of the active site and its flexibility

	Original	Case 1a	Case 2a	Case 2e	Case 3
d	13.45	23.22	20.70	8.57	16.71
s	0.007	0.020	0.050	0.002	0.110

While the optimized structures describe local minima near the geometry of the original conformation, they do not sufficiently represent the change in the flexibility of the structure of the enzyme. A number of quantities can be computed to follow the geometrical changes during the simulation. We chose a very simple one that measures the size of the active site. We calculated the average distance between the α-carbon atoms belonging to active-site residues Tyr7 and Tyr33, along with the standard deviation of this value during the simulation (omitting the results of the first 10 ps of the simulation). The distances (d) and the standard deviations (s) corresponding to some representative cases are tabulated below in angstrom (Å): (see Table 1).

One can see that in all cases the average distance of the Tyr7 and the Tyr33 residues differs significantly from the original value, which shows that the size of the active site (picturing a lock of the lock-and-key hypothesis) becomes larger (Cases I/a, II/a and III) or smaller (Case II/e) as a result of chirality change(s). In addition, the flexibility of the structure (described by s) grows in almost all cases, which indicates that the residues of the active site rarely have a chance to be in a catalytic active state. Interestingly, the change of the chirality in all active site residues makes the active site more compact and somewhat more rigid than in the original structure.

To illustrate some of the structures occurring during the simulation, we present three points (10, 50 and 100 ps after the start of the procedure, respectively). Structures are presented for the original enzyme and case I/a (Fig. 5), II/a and II/e (Fig. 6). The structures presented for the original enzyme show that both the molecule itself and its active site within it are confined to a limited region of the configurational space around the initial position. However, even in these chirality perturbed cases when the optimized

Fig. 5 Structures after 10, 50 and 100 picoseconds: the original molecule on the left hand side, Case I/a on the right hand side
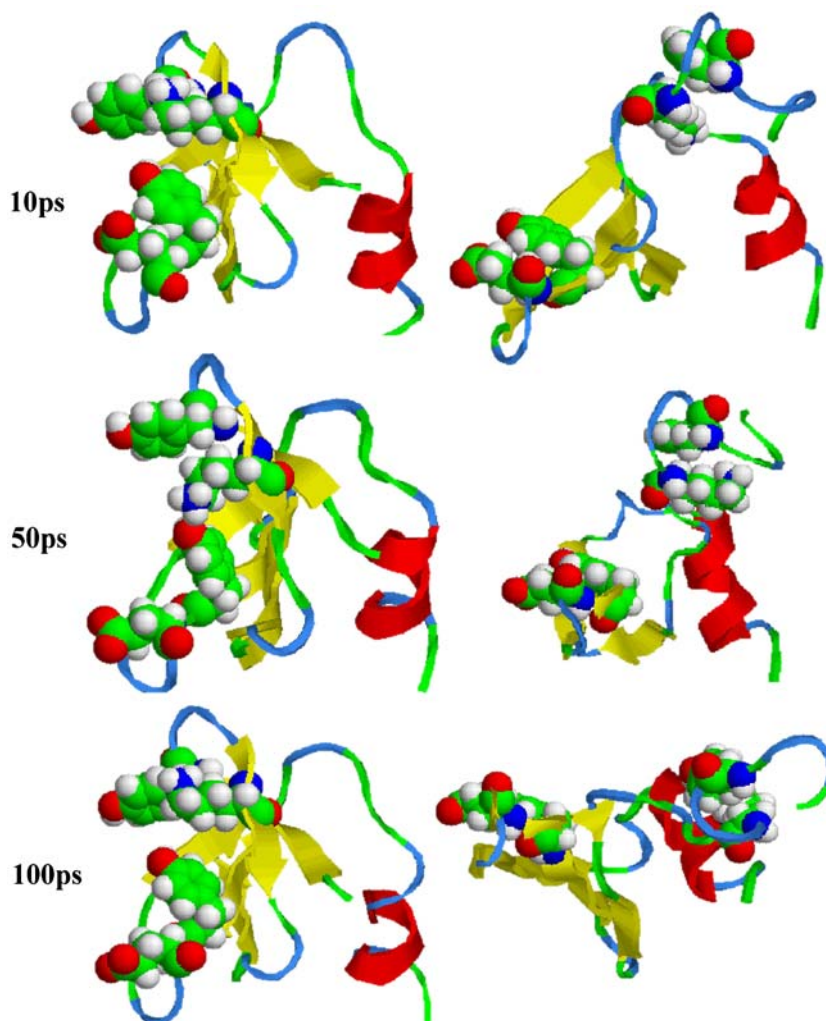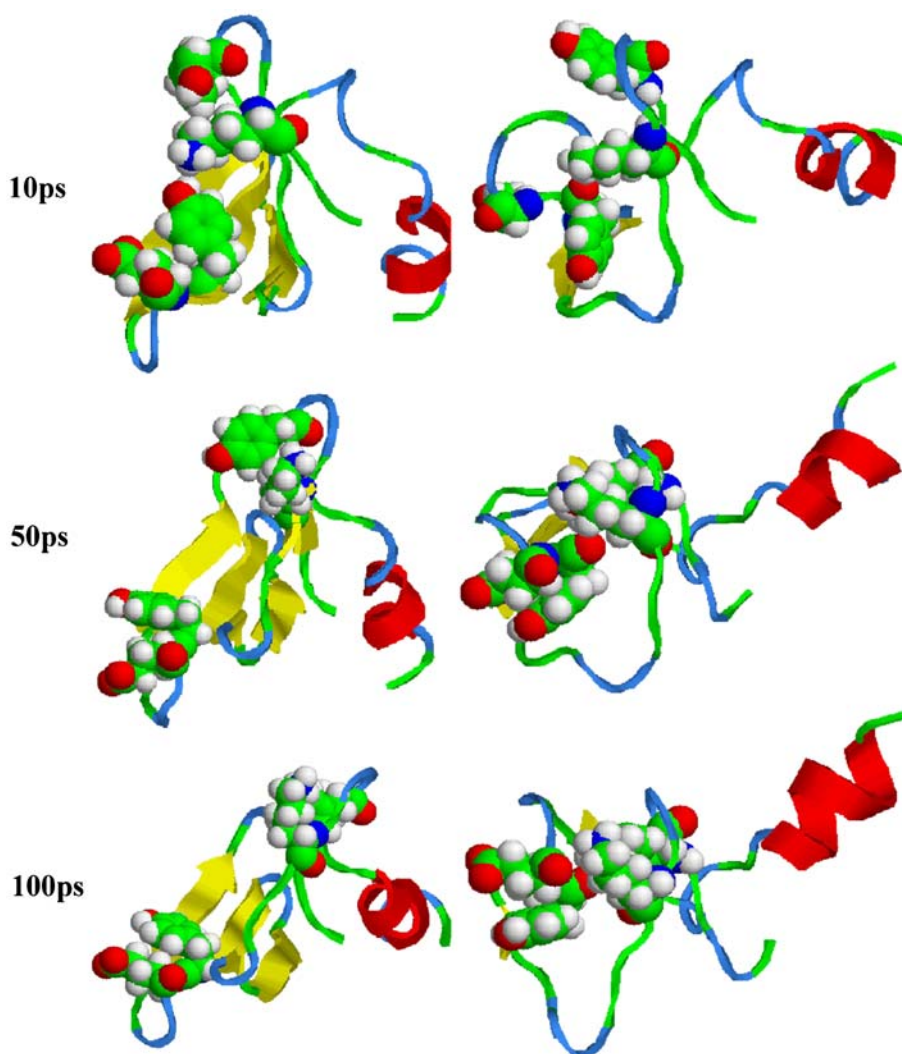
Fig. 6 Structures after 10, 50 and 100 picoseconds: Case II/a on the left hand side, Case II/e on the right hand side

10ps

50ps

100ps

structures show close resemblance to the conformation of the original enzyme, structures observed during the process of the simulation deviate far from their original positions. (In a longer simulation they may completely unfold due to the increased flexibility of the geometry.)

To summarize both the MM and the MD results, one can state that if a protein consists of a mixture of L- and D-amino acids (obtained either artificially or by epimerization), due to their changed conformation (as compared to the one composed of only L ones) it cannot catalyze the same biochemical reactions[2].

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[2] To underline this statement we should mention that the same proteins in their active and non-active form have different conductivities: the non-active form has the smaller conductivity [14, 15]. (For the different theoretical methods to calculate the frequency—dependent d.c. conductivity of these proteins see [16, 17]. Since most bio-catalytic reactions occur through charge transfer, this implies that their catalytic activity has to be diminished or disappears completely due to the change of the conformation of their active site.

Conclusion

It has been demonstrated on the example of Sso7d enzyme, by means of molecular dynamics simulation that changing the chirality of a number of residues alters the conformation of the active site of the enzyme, moreover it might significantly influence the overall secondary structure of the molecule. Thus enzymes containing D-amino acids would not be able to function properly. This most probably stands for any other (non-enzymatic) proteins as well. This modeling is the first detailed computational study of chirality perturbations to our knowledge.

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