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A mechanism for the stabilization of the secondary structure of a peptide by liquid ethylene glycol and its aqueous solutions

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Abstract

Molecular dynamics (MD) simulations have been performed to investigate the effects of ethylene glycol (EG) on the stabilization of the secondary structure of a polyserine helical peptide. Three model systems containing one peptide chain with initial helical structure and the following solvent components were considered: (a) 1379 SPC water molecules; (b) 1606 EG molecules; (c) EG/water mixture containing 1307 EG and 1978 water molecules performing a 40:60 composition. MD simulations show peptide stabilization in liquid EG and in an EG/water mixture but denaturation in aqueous solution. In the EG/water mixture water molecules on the first peptide solvation shell were displaced by EG ones. Structural analyses demonstrate that EG molecules are capable of forming multiple hydrogen bonds with the peptide side-chains, thus preventing the rupture of the intrapeptide hydrogen bonds. Therefore, the peptide stabilization is explained by the intercalation of EG molecules between its side-chains. This mechanism can also explain the stability of proteins secondary structure in solutions containing EG molecules as cosolvents.

Keywords: Peptide structure; Molecular dynamics; Solvation; Ethylene glycol

1. Introduction

The molecular mechanisms involved in folding and unfolding processes of proteins in solutions are not well understood, as well as the role that the solution composition plays in the changes observed in the secondary structure of proteins [1]. Several experimental evidences have been collected regarding the effects of solutes that may act either as a stabilizing agent or as a denaturing agent. For instance, trifluorethanol [2–5], some salts [6,7], sugars and polyols [8–18] are known to stabilize proteins in aqueous solutions, whereas guanidine hydrochloride [19,20] and urea [20–23] act as denaturing agents. Polyhydric alcohols and sugars are among the best stabilizing agents for proteins in aqueous solution and several theoretical mechanisms have been suggested to account for the protein conformational stability in the presence of these compounds, as follows: (1) they increase the surface free energy of water [11,12,14,17]; (2) there would be a preferential hydration of sugar and polyol molecules, decreasing the ability of the medium to break intramolecular protein hydrogen bonds [13,15]; and (3) the increase of dielectric constant [16]. These mechanism propositions are based on general assumptions but relevant discrepancies appear regarding the experimental behavior of some important class of solutes. The increase in the surface free energy of water is one of the most used hypotheses to explain the stabilization of proteins by polyhydric alcohols. However, polyols such as glycerol and ethylene glycol (EG) increase the protein conformational stability, although both of them decrease the surface tension of water [17,18].

The α-helix is the most common secondary structure in proteins [1] and is stabilized mainly by intramolecular hydrogen bonds but it seldom occurs in peptides in aqueous solution [24]. In the present work, we aim at explaining

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the stabilizing effect of EG and EG/water mixture on peptide structure. As the model peptide to be investigated is not supposed to be stable in aqueous solution, its position on a helix propensity scale should be analyzed. As discussed elsewhere, a helix propensity scale measure the contribution of a given amino acid to α-helix formation in proteins or peptides and several scales have been proposed. Pace and Scholtz [25] proposed a helix propensity scale relative to alanine and glycine amino acids attributing to alanine the highest helix propensity (value 0 Kcal/mol) and to glycine the lowest one, (value 1 kcal/mol). In this scale, the value attributed to serine amino acid is 0.50 kcal/mol. The intermediate position of serine amino acid in this helix propensity scale indicates a polyserine peptide as an adequate model to investigate the EG stabilizing mechanism. Therefore, in the present investigation a model peptide containing 16-serine residues with an initial α-helical structure was used. To the best of our knowledge, this is the first simulation of a peptide in explicit EG/water mixtures.

2. Computational details

The model peptide used in our simulations consisted of 16-serine residues in α-helix conformation and zwitterionic form. All simulations were conducted in the constant-NpT ensemble using the weak coupling scheme of Berendsen et al. [26] ($T = 298$ K and $\tau_T = 0.1$ ps; $p = 1$ bar, $\kappa = 4.5 \times 10^{-5}$ bar^{-1} and $\tau_p = 1$ ps). Standard periodic boundary conditions were considered, a 1.5 nm cut-off for the non-bonded interactions was used and the long-range correction taken into account by the particle mesh Ewald technique (PME) [27,28]. The motion equations were integrated using the leap-frog algorithm [29] with a time step of 1.0 fs. The potential energy surface was described using the OPLS-AA force field [30] for the peptide. and the flexible SPC model for water [31]. The gas-phase structures of EG molecules are accurately described by the OPLS-AA-SEI force field [32]. Scaling the OPLS-AA-SEI Lennard–Jones and Coulomb 1–4 interactions by factors of 0.65 and 0.60, respectively, a new set of parameters was obtained to accurately reproduce thermodynamical and structural properties of pure liquid EG and its aqueous solutions [33]. These parameters were used in the present work to model the EG molecules.

Three model systems containing one peptide chain and the following solvent components were considered: (a) 1379 SPC water molecules; (b) 1606 EG molecules; (c) EG/water mixture containing 1307 EG and 1978 water molecules performing a 40:60 composition. The starting structures of the peptide solutions were optimized by energy minimization runs using steepest descent and conjugated gradient algorithms to obtain an energy gradient below 100 kJ mol^{-1} nm^{-1}. The solvent molecules were allowed to relax for 300 ps keeping the peptide molecule rigid by means of a position restraint potential. The position restraints were removed and a production run of 10 ns was generated.

All calculations were performed using the GROMACS package (version 3.1.4) [34,35] on a 1.4 GHz AMD Athlon running Red Hat linux operational system (version 7.2).

3. Results and discussion

3.1. Structural parameter

The root-mean-square deviation (RMSD) as a function of time is reported in Fig. 1, where the peptide reference structure was the initial α-helix conformation, considering the whole peptide to compute the RMSD. The peptide secondary structure remains closer to its starting conformation in the pure EG and EG/water mixture than in pure water. The RMSD values in water increase steadily for *ca.* 1 ns, leveling off around 0.5 nm. In contrast, the peptide conformation is stable in the pure EG and EG/water mixture with the RMSD values leveling off around 0.1 and 0.15 nm, respectively.

The increase of the RMSD values in water result from the fraying of the C-terminal region of the helix, as indicated by the root-mean-square fluctuation (RMSF) per residue (Fig. 2), that shows a higher mobility of residues near the C-terminal region (residue 16). As expected from the RMSD values (Fig. 1), the RMSF values indicate an overall mobility of residues that is larger in water than in pure EG and EG/water mixture (Fig. 2). It should be noted that even in pure EG, where RMSF values attain their lowest level among the systems studied, the C-terminal region is more mobile, possibly indicating that this region plays an important role in the unfolding process of this peptide.

Fig. 3(a) and (b) show, respectively, the snapshots of the initial and the final peptide structure for each system studied.

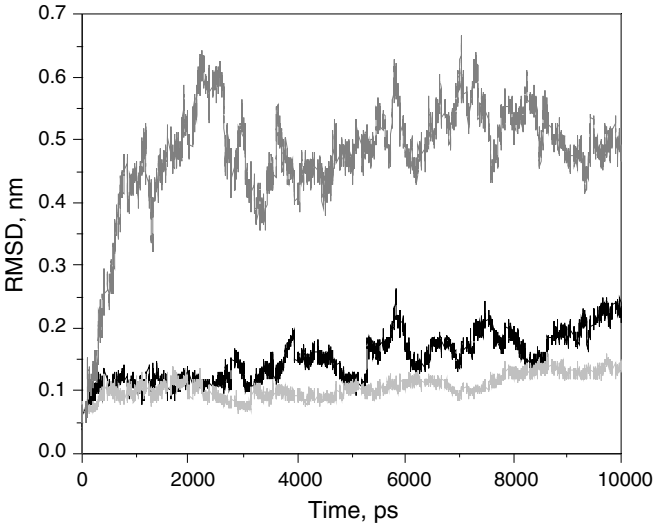

Fig. 1. The RMSD of the whole peptide from the initial structure. Water (dark gray); EG/water mixture (black) and EG pure (light gray).