EFFECT OF UREA AND TEMPERATURE ON THE MOLECULAR DYNAMICS OF BOVINE SERUM ALBUMIN IN HEAVY AND LIGHT WATER

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ABSTRACT

We report the effect of urea and temperature on the molecular dynamics of bovine serum albumin (BSA) in light and heavy water which was studied by computer simulation. It was observed that BSA was more stable in deuterium dioxide (heavy water) than ordinary water (light water). Consequently, the unfolding rate decreased because of the higher solvent dynamic viscosity of deuterium dioxide as compared to that of ordinary water. The dynamic viscosity of deuterium dioxide is 1.25 mPa.s compared to water which has a dynamic viscosity of 1.002 mPa.s at 20°C. We find that the rate of BSA unfolding is dependent on the viscosity of heavy and light water.

Keywords: molecular dynamics, BSA (bovine serum albumin), urea, simulation.

INTRODUCTION

The choice of light and heavy water is a prevalent issue in experimental and theoretical biochemistry [1]. For instance, samples for small angle neutron scattering (SANS) experiments are usually prepared by dissolving a definite specimen amount in a buffer solution of heavy water i.e. D2O instead of light water i.e. H2O. The use of D2O as a solvent instead of H2O provides better results in neutron scattering experiments since D2O has minimal effect on the sample but a dramatic effect on the scattering result due to the great penetrating power of neutrons and their sensitivity to the difference between hydrogen (H) and deuterium (D) in the sample solution. Furthermore, the study of hydrogel has shown a significant difference in the rate of gelation of BSA dissolved in D2O as compared to that dissolved in H2O. It is therefore pertinent to establish the differences in the dynamics of BSA in light and heavy water.

Molecular dynamics simulation is a vital tool in the study of protein unfolding process [2]. Due to computational power substantial increase, the molecular dynamics simulations, which make use of classical Newton mechanics to generate trajectories, are playing an ever-expanding role in biochemistry and biophysics. By adopting appropriate geometry, operating and boundary conditions, molecular dynamics simulations are capable of unraveling the protein folding or unfolding pathways.

Urea (CO(NH2)2) and heat are the most widely used protein denaturing agent [3]. Globular protein like bovine serum albumin denatures when heated in an aqueous solution. When heated above its denaturation temperature (typically 50°C - 80°C), it unfolds substantially. Urea destabilizes or perturbs the hydrophobic interactions and the hydrogen bonds in the protein [7], which results in loss of its biological functions. Therefore, it is important to understand this process and the resulting unfolding, in this case referring to BSA.
EXPERIMENTAL

High and low temperature unfolding molecular dynamics simulation of BSA was carried out in D\textsubscript{2}O and H\textsubscript{2}O in presence of urea as a denaturing agent using the rhombic dodecahedron box type. D\textsubscript{2}O solvent was modeled in GROMACS using GROMOS96 53a6 force field incorporated in GROMACS with the mass of hydrogen changed to deuterium in the SPCE file of the force field library. This practice was relatively new as GROMACS water models did not include deuterium dioxide solvent. However, it was approved (upon our request) by the software developers and researchers participating in GROMACS user mailing list.

RESULTS AND DISCUSSION

By changing the solvent mass (i.e. changing the mass of hydrogen to deuterium), we alter only the solvent viscosity and not the unfolding free energy. We have assumed that the interaction function would be the same as only the dynamic properties but not the thermodynamic one are affected by the masses.

The Stokes law asserts that the frictional drag exerted on a small particle moving through a fluid scales in proportion to the dynamic viscosity \( \eta \) of the solvent. This suggests that the relevant reaction friction for a polypeptide in a solvent is also proportional to \( \eta \). If the reaction friction \( \gamma \) varies directly with \( \eta \), then the modified Kramer’s equation implies a simple relationship between \( k \) and \( \eta \),

\[
k = \left( \frac{A}{\eta} \right) \exp \left( \frac{-\Delta G}{k_B T} \right)
\]

where \( k \) is the reaction rate, \( A \) is a constant that depends on the curvature of the free energy plot as a function of the reaction coordinate, while

\[\exp \left( \frac{-\Delta G}{k_B T} \right)\]

is the Arrhenius exponential term that relates the reaction rate to the temperature.

We deduce from Eq. 1 that the reaction rate decreases with increase of the solvent dynamic viscosity. The latter of D\textsubscript{2}O is 1.25 mPa.s at 20°C, while that of H\textsubscript{2}O is 1.002 mPa.s. The reaction rate decrease means decrease of the protein unfolding rate. Generally it is estimated on the ground of the radius of gyration of the protein atoms.

The plots in Fig. 1 below compare the radius of gyration of BSA dissolved in D\textsubscript{2}O and H\textsubscript{2}O at different temperatures. It is observed that at 25°C BSA is more compact in D\textsubscript{2}O than in H\textsubscript{2}O due to the frictional drag provided by the viscosity variation. At temperature increase to 80°C, BSA tends to unfold rapidly in D\textsubscript{2}O as much as in H\textsubscript{2}O. This is so because the viscosities of D\textsubscript{2}O and H\textsubscript{2}O both decrease at 80°C and approach close values, i.e. 0.4138 mPa.s and 0.3558 mPa.s, respectively. This is evident in the trend of the calculated radius of gyration of BSTAdissolved in D\textsubscript{2}O and H\textsubscript{2}O.

Effect of urea on BSA kinetics studied by molecular dynamics

The radius of gyration appears to increase significantly with urea concentration increase from zero to 5M, when compared with the effect observed in BSA dissolved in water free from urea. This trend is maintained at all temperature values studied.

Fig. 1. Radius of gyration vs. time at (a) 25°C; (b) 80°C.
CONCLUSIONS

We have used molecular dynamics simulation to study the unfolding kinetics of BSA in D$_2$O and H$_2$O. The difference in BSA radius of gyration can be attributed to the rate at which BSA unfolds in H$_2$O and D$_2$O considering the difference in their viscosities. The radius of gyration of BSA is used to measure their unfolding rates.

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