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Elucidation of Active Site Dynamics of Papain and the Effect of Encapsulation within Cationic and Anionic Reverse Micelles

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Abstract

In this study, steady state, solvation dynamics and rotational dynamics experiments have been carried out on a system of DACIA-tagged papain in bulk water and inside the water pool of cationic (cetyltrimethylammonium bromide, CTAB) and anionic (sodium bis(2ethylhexyl)sulfosuccianate, AOT) reverse micelles with varying water contents ($W_0 = 20$ to 50). While the absorption and emission maxima and the excited state lifetime did not show any noticeable change with the variation of the size of the reverse micelle, the change in solvation time, Stokes shift, rotational correlation time and residual anisotropy with the change in reverse micellar size were quite revealing. The average solvation time and Stokes shift of papain in bulk water are 0.22 ns and 125 cm⁻¹ respectively, which increase to 0.96 ns and 718 cm⁻¹ while inside CTAB reverse micelle of $W_0 = 20$. The solvation time and Stokes shift values decrease with the increase in the size of reverse micelle, approaching the corresponding values in bulk water when $W_0 = 50$. The solvation time and Stokes shift of the DACIA-tagged papain was found to be high while inside AOT reverse micelle also (0.47 ns and 438 cm⁻¹ respectively when $W_0 = 20$), but there was no monotonous variation with the change in size of micellar size as in the case with CTAB reverse micelle. From the anisotropy studies, it was seen that inside CTAB and AOT reverse micelles, there is a significant amount of residual anisotropy, which is absent in the case of DACIA-tagged papain in bulk water. The rotational correlation times were also found to be higher inside the reverse micelles than those in bulk water. Both residual anisotropy and rotational correlation time were found to be more in the case with AOT reverse micelle than with CTAB reverse micelle. These behaviours could be explained based on the electrostatic forces acting between the papain having a positive surface charge and the reverse micelles of cationic CTAB and anionic AOT.

Key words: Papain; solvation dynamics; time resolved fluorescence anisotropy; CTAB reverse micelle; AOT reverse micelle.

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