MA-7

Picosecond Protein Fluorescence and Time-Resolved Eu^{3+} Luminescence Spectroscopic Studies on the Roles of Ca^{2+} in Subtilisin Carlsberg

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Ca^{2+} is one of the most common metal ions associated with proteins, playing more or less well-defined functional roles in biological activities. In protease, the presence of Ca^{2+} slows down autolysis and enhances thermal stability. Subtilisin, one of the best studied proteases, is known to have two Ca^{2+}-binding sites. The kinetic profile of Eu^{3+} luminescence is decolvoluted to a double exponential decay. The faster component is ascribed to luminescence from Eu^{3+} bound to solvent water molecules and the slower one is due to luminescence from Eu^{3+} bound to the protein. This suggests that only one Eu^{3+} ion is bound to the protein. Time-resolved fluorescence of this single tryptophan protein shows a biexponential decay. Binding of metal cations to subtilisin Carlsberg increases the anisotropy and anisotropic decay time of tryptophan fluorescence very significantly. This implies that binding of metal cations triggers the conformational changes of the protein. The conformational changes by Ca^{2+}-binding increase the rigidity of the protein that is suggested to increase the chemical and thermal stabilities of the protease.