

High helical propensity of the β -lactoglobulin with non-native α -helical intermediate: a spectroscopic approach

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Abstract

It is generally considered that intermediates of protein folding contain partially formed native-like secondary structures. To understand the mechanism that stabilizes the non-native intermediate, it was characterized by circular dichroism the equilibrium unfolding transition of β -lactoglobulin induced by sodium dodecyl sulfate at pH 7. β -lactoglobulin is a predominantly β -sheet protein, although it has a markedly high intrinsic preference for α -helical structure. The far-UV CD spectrum of the intermediate, obtained by global fitting analysis of the CD spectra in the presence of various concentration of sodium dodecyl sulfate, was similar to the burst phase intermediate observed in the refolding thermodynamics and contained non-native α -helical structures. The effect of sodium dodecyl sulfate on the structure of native β -lactoglobulin at pH 7 was utilized to investigate the contribution of hydrophobic interactions to the stability of non-native α -helical intermediate. Addition of different concentrations of sodium dodecyl sulfate increased the helical content of the equilibrium intermediate, although the protein still assumed the native structure in the absence of sodium dodecyl sulfate. This indicates that because of the high helical preference of the amino acid sequence of β -lactoglobulin, the helical region protrudes into the boundary between the native and unfolded state, resulting in non-monomeric accumulation of the helical intermediate upon equilibrium unfolding of the native β -sheet structure. The present results suggest that a non-native α -helical intermediate accumulates during equilibrium unfolding of a predominantly β -sheet protein.

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