

Is Folding of β -Lactoglobulin Non-hierarchical? Intermediate with Native-like β -Sheet and Non-native α -Helix

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The refolding of β -lactoglobulin, a β -barrel protein consisting of β strands β A- β I and one major helix, is unusual because non-native α -helices are formed at the beginning of the process. We studied the refolding kinetics of bovine β -lactoglobulin A at pH 3 using the stopped-flow circular dichroism and manual H/²H exchange pulse labeling coupled with heteronuclear NMR. The protection pattern from the H/²H exchange of the native state indicated the presence of a stable hydrophobic core consisting of β F, β G and β H strands. The protection pattern of the kinetic intermediate obtained about one second after initiating the reaction was compared with that of the native state. In this relatively late kinetic intermediate, which still contains some non-native helical structure, the disulfide-bonded β -hairpin made up of β G and β H strands was formed, but the rest of the molecule was fluctuating, where the non-native α -helices may reside. Subsequently, the core β -sheet extends, accompanied by a further α -helix to β -sheet transition. Thus, the refolding of β -lactoglobulin exhibits two elements: the critical role of the core β -sheet is consistent with the hierarchic mechanism, whereas the α -helix to β -sheet transition suggests the non-hierarchic mechanism.

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