

The existence of the dissociation stage in the heat-induced aggregation of bovine liver glutamate dehydrogenase

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The detailed mechanism of heat-induced aggregation of bovine liver glutamate dehydrogenase (GDH) has been studied. The initial stages of the aggregation process at 50 °C were investigated using analytical ultracentrifugation. The sedimentation velocity analysis showed that the native protein at the concentration of 0.2 mg/mL contained hexameric GDH and larger native associates. The samples preheated at 50 °C during 3 min contained the oligomeric forms of larger size than in the native sample, along with hexameric GDH. Further heating (5 min) produced the dissociated forms smaller than hexamers, although the previous oligomers and the native protein remained. The thermal treatment up to 10 min resulted in the loss of 83% of protein due to precipitation of large aggregates. These data allowed us to refine the mechanism of the heat-induced GDH aggregation studied in our laboratory. GDH denatures upon heating with the formation of molten globule-like intermediate [1]. These minor structural changes are apparently enough to initiate protein aggregation. The irreversible dissociation step occurs after unfolding. The further growth of aggregates proceeds by attachment of dissociated forms to the existing associates and by the aggregate-aggregate sticking.

These data clarify the mechanism of GDH aggregation and directly confirm the existence of the protein dissociated forms after its denaturation at the elevated temperatures. The specified information about GDH aggregation and stability can be useful in developing of GDH-based biosensors and the studies of GDH function in vivo under thermal stress conditions.

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1 Singh N, Liu Z & Fisher HF (1996) The existence of a hexameric intermediate with molten-globule-like properties in the thermal denaturation of bovine-liver glutamate dehydrogenase. *Biophys Chem* 63, 27–36.

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