Reductive alkylation causes the formation of a molten globule-like intermediate structure in geobacillus zalihae strain T1 thermostable lipase.

ABSTRACT

A thermostable lipase from Geobacillus zalihae strain T1 was chemically modified using propionaldehyde via reductive alkylation. The targeted alkylation sites were lysines, in which T1 lipase possessed 11 residues. Far-UV circular dichroism (CD) spectra of both native and alkylated enzyme showed a similar broad minimum between 208 and 222 nm, thus suggesting a substantial amount of secondary structures in modified enzyme, as compared with the corresponding native enzyme. The hydrolytic activity of the modified enzymes dropped drastically by nearly 15-fold upon chemical modification, despite both the native and modified form showed distinctive α-helical bands at 208 and 222 nm in CD spectra, leading us to the hypothesis of formation of a molten globule (MG)-like structure. As cooperative unfolding transitions were observed, the modified lipase was distinguished from the native state, in which the former possessed a denaturation temperature (T m) in lower temperature range at 61 °C while the latter at 68 °C. This was further supported by 8-anilino-1-naphthalenesulfonic acid (ANS) probed fluorescence which indicated higher exposure of hydrophobic residues, consequential of chemical modification. Based on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis, a small number of lysine residues were confirmed to be alkylated.

Keyword: Chemical modification; Reductive alkylation; Circular dichroism; Thermostable lipase; Molten globule.