Chemical and acidic denaturation of a homodimeric glutathione transferase mu class from *Rhipicephalus (Boophilus) annulatus*

Abdel-Monem Abdalla[1]*, Yasser Shahein[1], Yousry M. Hamdy[2], Amr E. El-Hakim[1].and Ragaa Hamed[1]

[1]Molecular Biology Department, National Research Centre, Dokki, Giza, Egypt.

[2]Spectroscopy Department, National Research Centre, Dokki, Giza, Egypt.

*To whom correspondence should be addressed,
Fax: +20233370931, Phone: +20101876762.

E-mail address: abdel_monem@hotmail.com

Abstract

The equilibrium unfolding of a mu class glutathione transferase from *Rhipicephalus (Boophilus) annulatus* (*Ba*GSTM) has been performed using guanidinium chloride (GdmCl), urea and acid denaturation to investigate the unfolding intermediates. Protein transitions were monitored by intrinsic fluorescence and 8-anilino-1-naphthalenesulfonate (ANS) binding. The results indicate that unfolding of *Ba*GSTM using GdmCl (0-4.0 M) is a multi-step process, i.e., at least two intermediates coexist in equilibrium. The first intermediate, a partially dissociated dimer, exists at low GdmCl concentration (less than 1.5 M). This intermediate undergoes dissociation into two monomers at GdmCl concentration between 1.5 and 2.0 M. The monomeric intermediate started to be completely unfolded at higher GdmCl concentrations (> 2.0 M). Unfolding using urea (0-8.0 M) and acid-induced structures as well as the ANS fluorescence in presence of different concentrations of GdmCl or urea confirmed that the unfolding is a multi-step process. The formation of a molten globule state (a monomeric intermediate) at pH less than 3.8 was suggested by the strong enhancement of fluorescence of ANS and protein concentration dependent.

Keywords: Glutathione transferase; *Boophilus annulatus*; Fluorescence; Folding; Unfolding; Subunit interactions; Intermediates.