Conformational stability and folding of human class Omega GST O1-1

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Glutathione transferases are an ancient superfamily of proteins that are thought to have evolved from a thioredoxin-like ancestor in response to oxidative stress. These enzymes play a role in phase II detoxification, by catalysing the conjugation of a variety of electrophilic compounds to water-soluble glutathione (GSH). Omega is one of the ancient members of this family of proteins and has recently been discovered. Equilibrium unfolding techniques have been used to determine the conformational stability of GST O1-1, using guanidinium chloride as a chemical denaturant. The intrinsic tryptophan fluorescence spectroscopy and far-UV circular dichroism were used as spectroscopic probes. The unfolding transitions are monophasic and superimposable, meaning that the secondary and tertiary structural changes occur simultaneously as the protein unfolds. The data-fitting to a two-state model gave a ΔG_{H2O} of 16 kcal/mol and the m-value of 3.8 kcal/mol/M guanidinium chloride. The unfolding process of human GST O1-1 is possibly a three-state process, ie. from the native dimeric protein to two unfolded monomers via two nativelike monomers. The unfolding kinetics studies of GST O1-1 were performed using stopped-flow methods and fluorescence as a probe. Data analysis reveals that the kinetic unfolding event of GST O1-1 is a single phase. The dependence of the apparent unfolding rates on guanidinium chloride fits to a linear regression and gives the m_u-value of 1.4 kcal/mol/M guanidinium chloride. The ratio of m_u/m is 0.37 and is an indication that the accessible surface area of the transient intermediate resembles that of the native protein.