

## **CONFORMATIONAL DYNAMICS AND FOLDING KINETICS OF SOLUBLE CLIC1**

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CLIC1 is a monomeric member of the glutathione transferase (GST) family that can exist in both soluble and membrane forms, where it functions as a chloride ion channel. Not much is known about the structure of the transmembrane form or the manner by which it forms a channel. Studies of the changes in structure and stability that soluble CLIC1 undergoes as it approaches the membrane may provide insight to the mechanisms of CLIC1 membrane insertion and channel structure. HPLC was used to determine the molecular sizes of the CLIC1 forms. Undialyzed and fresh monomer had the same sizes, however, the undialyzed form had a shoulder corresponding to 63.4 KDa that could be indicative of some dimeric species being formed under non-reducing conditions. It was also noted that the size of the dimer was not affected by differing salt concentrations. Unfolding kinetics experiments showed the dimeric form unfolding much quicker than the monomeric forms. While the monomer took approximately 17 minutes to unfold, the dimer unfolded in under 10 minutes. Stopped-flow kinetics experiments were performed in order to monitor the early events of unfolding. A fast phase of unfolding was observed that occurred within 0.1 seconds. Refolding kinetics showed CLIC1 to fold within 400 seconds with the slow phase exhibiting a time constant of 103 seconds. A burst phase was also observed. Compared to other monomeric members of the GST family CLIC1 appears to unfold relatively slowly, while refolding occurs at a much quicker rate.