Title: Biophysical studies of the structure and backbone dynamics of gsPGK using NMR relaxation methods.

ABSTRACT

The backbone dynamics of the full length and the N-domain of *geobacillus stearothermophillus* phosphoglycerate kinase (gsPGK) have been characterised using ^{15}N relaxation and relaxation dispersion measurements. The V I42C mutation of NPGK induced unexpected decrease in the denaturant dependence of the free energy between the folded and unfolded states (m-values) (Cliff, *et al.*. 2006). This decrease in m-values of the V142C NPGK cannot be ascribed to a change in the backbone dynamics. Backbone conformational dynamics in the free and bound forms of gsPGK were recorded. Comparison of the fast time scale dynamics (pico-nanosecond) of the free and the bound (ADP complexes) forms of gsPGK show a significant increase in the backbone dynamics upon the ADP binding. This increase in dynamics is global. The increase in dynamics caused by ADP binding is reversed by the formation of transition state analogue (TSA) complex. These results are interpreted in the context of the structural tightening hypothesis, which predicts shorter, less dynamic, hydrogen bonding in TS-bound states compared to substrate-bound states of enzymes. In particular, the recorded changes in dynamics of the ADP binding site residues of gsPGK and their hydrogen bond network show a strong correlation with the changes in amide proton shifts, which are strongly affected by hydrogen bond length. Relaxation dispersion experiments detect slow (millisecond) timescale dynamics and conformational exchange in the ADP and 3-phosphoglycerate (3PG) complexes of *gsPGK*. The induced chemical shifts changes associated with ms dynamics in the

ADP complex do not correlate with the amide nitrogen shifts changes between the ground states of the ADP and TSA complexes, which indicates that the conformational exchange rate does not reflect the complete opening and closing process of gsPGK. These studies have provided a reasonably complete picture of how gsPGK responds to structural and dynamics changes during its catalytic cycle.