Large Thermal Fluctuations in the Tertiary Structure of Methemoglobin

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The presence and the large amplitude of tertiary fluctuations in the heme pocket of MetHb are demonstrated by our results concerning an intramolecular reaction equilibrium.

The millisecond relaxation of MetHb dimers at pH 6, 5-20°C in aqueous solution was investigated by T-jump technique and assigned to a first-order reaction with $\Delta H=(-645 \pm 5)$kJ/mol, $\Delta S=(-275 \pm 20)$J/mol K, and K=0.0055 - 0.0013. It must be interpreted as binding of N_{ε} of His E7 to the heme iron due to energetic, spectroscopic, and kinetic (k_{off}) reasons arguing against a different possible reaction mechanism. Inspection of the known X-ray atomic coordinates shows that the shift of 2-3 Å required for the reaction is based on a motion of the helix structure since internal rotations of the His residue cannot lower the distance N_{ε}-Fe. Fluctuations of this type may be important for the oxygen binding mechanism.

The role of entropy in the framework of protein structure and function is stressed by the fact that the intramolecular binding is supressed by the large negative reaction entropy.