

P010 Hydrostatic pressure: a new probe of promoting motions in enzyme catalysis

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The role of protein dynamics during enzyme catalysis is of current interest. Transition state theory dictates that dynamical processes that alter the reaction barrier height will consequently alter the rate of reaction. Conversely, hydrogen tunnelling reactions – those with a considerable quantum mechanical contribution to the transfer – are particularly sensitive to changes in the width rather than the height of the barrier. In a combined experimental and theoretical approach, we have begun to use hydrostatic pressure to probe enzymatic hydrogen tunnelling reactions. The aim is to use pressure to mimic those dynamical processes that cause barrier compression – so called promoting motions. We have now examined the effect of pressure on two enzymatic H-tunnelling reactions that are thought to be assisted by promoting motions: the reduction of the flavoenzyme morphinone reductase (MR) by NADH and the reduction of the quinoprotein aromatic amine dehydrogenase (AADH) by phenylethylamine. Both the observed rate and H/D kinetic isotope effect (KIE) of the MR reaction increase substantially with pressure, while conversely both the rate and KIE of the AADH reaction decrease with pressure. We can now rationalise these observations in terms of an H-transfer model where both the transfer distance (barrier width) and the frequency of the promoting motion are sensitive to pressure.