Activation volumes and structural reorganisation in the conformational exchange of β-phosphoglucomutase transition state analogue complexes

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Order naturally turns to disorder, and living beings are in a constant fight against this tendency. One of the ways biological systems achieve order is by exploiting the phosphoester bond, which is remarkably stable and can last for millions of years. Biology manipulates these extremely strong bonds to store energy and drive otherwise unfavorable reactions. This is achieved by enzymes, proteins (biological building blocks composed of amino acids) that aid chemical reactions.

Enzymes help reactions happen through several strategies, such as by orienting atoms correctly, increasing the reactivity of specific atoms, or stabilizing highly energetic states. They are flexible and dynamic structures that expand, contract, and move around to perform their function. They exist as many different conformations and constantly switch between them. Observing the movement of these enzymes is a key to understand how they work.

Here we examine β-phosphoglucomutase, an enzyme that breaks a phosphoester bond from one position of a sugar and reforms this bond at a different position, in what we call a phosphoryl transfer reaction. The exact way β-phosphoglucomutase works is still unclear. For the reaction to happen, the sugar needs to be released in an intermediate form and rebound by the enzyme. The subject of ligand release (ligand is a general term for a molecule that binds to an enzyme) itself has not gained much attention as enzymology studies often focus on the importance of ligand binding rather than release.

We aim to observe the structural changes happening in β-phosphoglucomutase which allow it to “kick out” the intermediate sugar from the reaction core of the enzyme. Nuclear magnetic resonance, *nuclear* referring to the nucleus of the atom, is one of the ways we can observe the dynamic processes of an enzyme as it moves around in solution as it would inside the cell. Since each nucleus of an atom is a tiny magnet, we can observe their signals by putting them in a very strong magnetic field. Then, we sing to these nuclei by using specialized pulses and listen to what they sing back to us, which gives us atomic-level information.

We have trapped the enzyme in a mimic of the highest energetic step of the phosphoryl transfer reaction. By compressing the enzyme using high pressures we were able to measure the change in volume between two states of this energetic step and identify which specific regions of the enzyme are involved in this change. We observed a volume change corresponding to approximately two water molecules, which may be linked to the movement that triggers ligand release. We also observed a partial opening of the reaction center that is buried within the enzyme. This gives us another piece of the puzzle needed to uncover the mechanism of β-phosphoglucomutase.

While β-phosphoglucomutase is a specific system, phosphoester chemistry is central to many biological processes, including DNA metabolism and cellular signalling. These findings improve our understanding of how phosphoenzymes work. Additionally, what we have observed is likely relevant to the general mechanism of ligand release, an important step in all enzymatic processes and protein-ligand interactions. In the future, this knowledge may help guide the design of novel enzymes and therapeutics.