An Advanced Protocol for Expression of Perdeuterated Large Proteins and Dynamic NMR Studies of Loop Motions in 53 kDa Triosephosphate Isomerase

by

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ABSTRACT

Nucleic magnetic resonance (NMR) is a powerful tool to characterize three-dimensional (3D) structures and dynamics of proteins and protein-ligand complexes. However, large proteins (>20-30 kDa) yield crowded spectra with significant spectral overlap due the greater number of inherent peaks, and also to fast spin relaxation which uniformly broadens the NMR peaks. The spectral crowding may sometimes be alleviated by subunit-specific or segmental labeling of a protein. In addition, uniform ^{2}H labeling (deuteration) is a key for reducing spin relaxation and therefore overlap. Either approach requires the preparation of a well-folded NMR sample of sufficient purity and concentration. Significant improvements can also be made through sophisticated pulse sequences that further reduce spin relaxation rates.

In this thesis, an advanced cell-adaptation protocol for perdeuterated protein expression is provided. The approach provides cells with enhanced proliferation and expression abilities from deuterated minimal media. This approach was applied to human adult hemoglobin (Hb A), a 64 kDa, tetrameric protein that requires significant post-expression processing. Using the protocol, JM109(DE3) *E.coli* were specially adapted to 99% deuterated environment including careful accounting for any residual protons from buffer sources, added glucose and other reagents. As a result, the yield of expressed Hb A was 2.5-fold higher than that obtained from cells adapted to a deuterated environment using more-traditional methods. In addition, significant improvement on deuteration level (17% plus) was achieved. This approach utilizes only simple shake-flask growth and expression cultures. While potentially also of use for more complex schemes based in fermentation, the reliance on shake flasks avoids any need for additional expertise and costly equipment for fermentation. Furthermore, shake-flask expression is amenable to arbitrarily small expression volumes, whereas many fermentors require a 3-10 L minimum. Avoiding such volumes when expressing from deuterated and otherwise isotopically enriched media can also greatly reduce costs. The adaptation protocol was tested with both JM109 and JM109(DE3) *E.coli*, and with pre- and post-transformation with the Hb A expression plasmid (pHE7). The (DE3) strain consistently outperformed its parent strain in response to adaptation, with the latter failing to survive adaptation in multiple trials. Furthermore, pre-transformed cells were

consistently more receptive to adaptation. Finally, methods for purification and other post-processing of HbA were detailed, which are essential for NMR structural and dynamic studies on Hb A.

This thesis also presents a dynamic study on chicken triosephosphate isomerase (cTIM) using a TROSY (Transverse relaxation optimized spectroscopy)-selected, off-resonance, spin-locked relaxation-dispersion experiment ($R_{1\rho}^{\beta}$). The loop 6 motions between open and closed conformations were probed through the variations in the spin-locked relaxation rate ($R_{1\rho}^{\beta}$) as a function of strength of an off-resonance spin-lock field (B_1^{lock}). This spin-relaxation probe can quantify motions on the timescale (~8,000 s^{-1}) relevant to TIM catalysis, which is rate-limited by the loop-6 motion. Experiments here explore changes in the motion as the enzyme progresses along the catalytic reaction coordinate by following the open/close equilibrium in both apo and ligand-bound states, where the latter mimics the enzyme-transition-state complex. Results from apo cTIM at V167 in the C-terminal hinge of loop 6 revealed expected loop motion at the catalytic timescale. However, results here show that loop motions in the noted bound form occur on a very different timescale, likely much slower than that of the apo form. This may serve to facilitate catalysis by (a) slowing conversion from the catalytically active, closed form until the chemical steps of catalysis are complete, and (b) preventing premature escape of the transition state ligand, which, if allowed, is known to result in generation of a toxic side product.