SUPPORTING INFORMATION

Mechanism for Activation of Triosephosphate Isomerase by Phosphite Dianion: The Role of a Ligand-Driven Conformational Change

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Preparation of L232A Mutant TIM from *Trypanosoma brucei brucei*. The plasmid pTIM containing the wildtype gene for TIM from *Trypanosoma brucei brucei* (*Tbb* TIM) was a generous gift from Professor Rik Wierenga. Site-directed mutagenesis to introduce the L232A mutation was conducted using *Pfu* Ultrahigh Fidelity DNA polymerase following the Stratagene protocol. The primer used to introduce the L232A mutation, in which the altered codon is underlined, was: 5′-CGA-GAC-GTC-AAC-GGC-TTC-GCT-GTT-GGT-GGT-GCC-TCA-CTG-AAG-C-3′.

After completion of the PCR, 20 units of the restriction enzyme *Dpn*I were added to 30 μL of the reaction mixture followed by incubation for 1 h at 37 °C, in order to degrade the methylated DNA template. *E. coli* strain K802 was transformed with 1 μL of the *Dpn*I-digested PCR product. Several colonies containing possible mutants were picked, and the plasmid DNA was purified using the QIAprep Miniprep Kit from Qiagen. The presence of the gene for L232A mutant *Tbb* TIM was verified by DNA Sequencing at the Roswell Park Cancer Institute (Buffalo, NY).

L232A *Tbb* TIM was overexpressed in *E. coli* BL21 pLysS grown in LB medium at 18 ˚C. The protein was purified by ammonium sulfate precipitation followed by gradient elution on CM Sepharose cation exchanger, following a published protocol.[1] The enzyme obtained from the column was judged to be homogenous by gel electrophoresis. The concentration of L232A mutant TIM was determined from the absorbance at 280 nm using an extinction coefficient of 3.5×10^4 $M^{-1}cm^{-1}$ that was calculated using the ProtParam tool available on the Expasy server.[2,3]

REFERENCES

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