ABSTRACT

The genome search of *Thermococcus kodakaraensis* revealed three open reading frames, *Tk0304, Tk1299* and *Tk1392*, annotated as NADH oxidases. Two of the three open reading frames (*Tk1299*and *Tk1392*) have already been cloned and proved to be true NADH oxidases. Present study deals with cloning, sequencing and expression analysis of *Tk 0304.*

Tk 0304 is composed of 1320 nucleotides. It has been cloned and expressed in *E. coli* (BL-21 codon plus). NADH oxidase/0304 is composed of 439 amino acids, with a calculated molecular weight of 47kDa. NADH oxidase/0304 was over expressed in the absence of induction at 37 ^{0}C. The recombinant enzyme was purified by heat treatment at 80 ^{0}C, which as a result of thorough centrifugation at 13,000 rpm, removed almost all of the debris proteins of the host. Further purification was achieved by AKTA low pressure chromatography system (FPLC) from Pharmacia Biotech. Resource Q (Amersham Biosciences), which was a strong ion exchange column, prepacked with 15µm beads of polystyrene/divinyl benzene, having column volume of 6ml was used for this purpose. The purified enzyme showed a prominent band on 12.5% SDS-PAGE, with a molecular weight of 47kDa. It has been observed that FAD acts as a cofactor of NADH oxidase/ 0304 at 50 ^{0}C, but further increase in temperature makes the enzyme FAD independent. The enzyme is most active at pH 9.0 and is stable up to 100 ^{0}C, without any loss of activity. Treatment of the enzyme with organic solvents (ethanol and DMSO) results in a slight increase in activity, but after crossing the limit of 30% activity is reduced to half than that of the original values. Current analysis shows that $AgNO_3$ remarkably increases the activity of Tk 0304. It has been observed that the enzyme looses half of its activity in the presence

of 15mM KCN. Detergents such as Tween 20, Triton X-100 and SDS have no effect on the enzyme. Salts of mono-valant metals (NaCl and LiCl) increase activity of the enzyme, when their concentration in the solution reaches 300mM. Protein concentration in the purified fraction of Tk 0304, obtained from Resource Q column of AKTA FPLC system, was determined by Bradford method. Calibration standard used for the assay was BSA (Bovine Serum Albumin) with stock concentration 0.2mg/ ml (BIO RED). Kinetic studies of the purified fraction of NADH Oxidase/ Tk 0304 were performed by using different concentrations (40-80μM) of the substrate (NADH), with the fixed amount of enzyme (5μl) in each assay. Other reagents used in the assay were 50mM Tris (pH 9) and 5mM EDTA at 50 ^{0}C.

The present work adds useful information about the NADH oxidases in hyperthermophilic archaea, in general, and *T. kodakaraensis* in particular.