ABSTRACT

The present work is concerned with purification and characterization of previously cloned recombinant alpha amylase from *Thermotoga petrophila.* Expression of the recombinant enzyme was checked at different intervals with 0.5mM IPTG concentration. Maximum expression was obtained 24 h after induction. Heat treatment of crude enzyme at 70° and 80°C for 1-2 h was carried out but maximum *E. coli* protein denaturation at 80°C after 2 h heat treatment was observed. Ion exchange chromatography was used to purify the heat treated sample. Active fractions were analyzed with standard protein marker on SDS-PAGE. A single band of 47 kD appeared on gel was obtained. Optimum activity of the recombinant enzyme was maximum at 90°C with citrate phosphate buffer (pH 6). Purified enzyme showed its maximum specificity toward 1.2 % soluble starch with 10 min incubation. Alpha amylase remained fully stable up to 80°C even after 4 h incubation. However, denaturation of enzyme was observed at 90° and 100°C after 1 h. Among the different metal ions investigated, Ca^{+2} ions markedly increased enzyme activity upto 90% whereas Co^{+2}, Ni^{+2} and Pb^{+2} reduced activity upto 72, 28 and 22% respectively. EDTA and urea were strong inhibitors of recombinant protein. Presence of SDS, Tween 80 and Beta mercaptoethanol along with different organic solvents made the enzyme less active under optimum conditions. Purified α-amylase remained stable at 4°C and in room temperature up to three weeks. A very slight decreased in activity was observed in fourth week. Maltose was the end product as analyzed by TLC.