Cysticercosis by Taenia solium is an important disease caused by the presence of larval stage in swine and human, can produce several clinical manifestation including death. In México 10% of medical attention in neurological disease is because cysticercosis. The lack of economical resources and poor hygiene in the communities, difficult the implantation of preventive methods. However molecules capable to induce protection against helminths have been proposed, for example, the Glutathione S-Transferase (GST), the Triose phosphate isomerase (TPI) and the Glyceraldehyde 3-phosphate dehydrogenase (GAPDH). These enzymes are vaccine candidates against schistosomiasis. The aim of this study is the partial characterization of Taenia solium for future use in vaccination assays. cDNA clones of TPI and GAPDH isolated from a library, encodes a protein of 250 and 336 amino acids respectively. Amino acids sequences have a high homology with others reported enzymes. The Taenia TPI had conserved catalytic site amino acids and structure characteristic of this enzymes with 8 domains alpha-beta. The cDNAs coding to the enzymes were cloned into the expression vector pRSET to produce the recombinant enzymes. The recombinant TPI and GAPDH shown a molecular mass of 27 kDa and 35 kDa respectively determined by SDS-PAGE. The recombinant TPI has catalytic activity and preliminary vaccination assays with this enzyme promoted 55% of parasite reduction in a cysticercosis murine model.

Purification and kinetic properties of catalase from Salmonella typhimurium and Paracoccus denitrificans

E. Keyhani(1), Sh. Golikho(2), T. Lasgarbolloki-Livan(1), A. Nezami-Ghorbani(1)

(1) Inst. Biochem. Biophys., Univ. of Tehran, P.O.Box 13145-1384, and
(2) Azzahra University, Tehran, Iran

Catalase was purified from Salmonella typhimurium (S.t.) and Paracoccus denitrificans (P.d.). In S.t. the oxidized catalase showed an absorption spectrum with maxima at 405, 540, 586 and 629 nm while in P.d. the absorption bands of the oxidized catalase were at 410, 502, 544 and 626 nm. Characterization of catalase activity, including optimum pH, Km and k determinations were done by monitoring the decrease in absorbance of H2O2 at 240 nm. S.t. had two distinct active catalases. One which exhibited catalase activity only had a pH optimum of 7.0 with K_m=34.5 mM, k=1.8X10^{-3} sec^{-1} and K_cat=940 l/min. The other had both catalase and peroxidase activities. The catalase activity was optimum at pH 6.0 with K_m=2.2 mM, k=1X10^{-1} sec^{-1} and K_cat=130 l/min; the peroxidase activity, monitored at 460 nm with 0.3 mM o-dianisidine and 2 mM H2O2 as substrates, was optimum at pH 5.0 with K_m=1.3 mM. In contrast to S.t., P.d. exhibited only one enzyme with catalase activity, having an optimum pH at 7.0 with K_m=2 mm, k=1.8X10^{-3} sec^{-1} and K_cat=205 l/min. Data suggested that in S.t. there were both a catalase and a peroxidase, like the system previously reported for E. coli. In contrast, in P.d. there was only one catalase. Moreover, the values of k (sec^{-1}) suggested that, at pH 7.0, S.t. catalase was 53 times more active than P.d. catalase.

The Role of the C-terminus in Bacillus subtilis Inorganic Pyrophosphatase Activity

M. A. Konopka, T. W. Young
University of Birmingham, School of Biosciences, Edgbaston B15 2TT, Birmingham, UK

Bacillus subtilis inorganic pyrophosphatase is the first recognized representative of a newly identified Family 11 of Inorganic Pyrophosphatases. These enzymes bear no sequence similarity to most of the currently known inorganic pyrophosphatases but show a high degree of sequence similarity among their own group. One of the most prominent regions of conservation in all members of the family is found near the C-terminus. We have examined the role of the C-terminus including this conserved 'signature' assembly by using Polymerase Chain Reaction to obtain truncated proteins. Two enzyme variants were produced with deletions removing 5 and 17 C-terminal amino acids, respectively. The 17 amino acid deletion mutant had no activity under the conditions tested, whereas the activity of 5 amino acid truncated mutant was seriously impaired with at least a sixfold decrease in specific activity. Gel permeation chromatography of the non-truncated and two truncated proteins showed them to have relative molecular masses consistent with a tetrameric structure. The C-terminus is clearly essential for enzyme activity. Removal of the non-conserved 5 amino acids causes a significant reduction in activity. Removal of 17 amino acids including the 'signature' sequence totally inactivates the protein. The results imply a role of the C-terminus in maintaining active site conformation rather than oligomeric subunit composition.