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Biochemical properties of the deglycosilated recombinant invertase from *Candida guilliermondii* MpIIIa expressed on *Pichia pastoris*

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Enzymes are highly effective biological catalysts with great industrial importance because of its versatility and action specificity. It has been reported that glycosylation plays a key role in the biochemical properties of enzymes. The native invertase INV3a-N from *Candida guilliermondii* MpIIIa is a glycoprotein with a carbohydrate content of ~74%, with optimal activity at pH 5.0 and 65°C. In order to improve our knowledge on the crucial role of glycosylation on the biochemical properties of enzymes, the gene inv3a encoding the invertase INV3a-N from *C. guilliermondii* MpIIIa was expressed in *Pichia pastoris* X-33, an expression system well known by its low glycosylation of recombinant enzymes. The *inv*3a gene presented an ORF of 1545 nucleotides encoding a polypeptide of 514 amino acid residues, with a theoretical MW of 58 kDa. The inv3a gene was extracellularly and constitutively expressed in *P. pastoris*. The recombinant enzyme, named INV3a-R, presented an estimated MW of 66 kDa with a carbohydrate content of ~4-5%, and optimal activity at pH 6.0 and 50°C. Interestingly, INV3a-R displays a dramatic diminution in thermal stability at 50°C (98.75%), substrate inhibition (50%), V_{max} (70%), optimal temperature (23%) and substrate affinity (900%) compared to those values observed for INV3a-N. However, despite being a thermolabile enzyme, INV3a-R is active at pH values up to 6.0, which represents an interesting feature for application in industrial confectionery substrates, whose pH is near 7.0. Findings here confirm the crucial role of glycosylation in the biochemical properties of the invertase INV3a-N from *C. guilliermondii* MpIIIa.

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Occupational risk assessment of oxidative stress and DNA damage in electroplating workers exposed to chromium

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Chromium is extensively used in electroplating industry which appears to be associated with genetic damages in workers. Therefore, the present study was undertaken to investigate the oxidative stress parameters and DNA damage in chromium exposed tanners. DNA damage in lymphocytes was measured by the comet assay. The results showed that blood chromium, DNA damage, superoxide dismutase (SOD) and malondialdehyde (MDA) levels were significantly higher ($p<0.001$), while glutathione (GSH) level was significantly lower ($p<0.001$) in exposed groups as compared to control group. In Pearson correlation analysis, blood chromium level showed significant correlation with oxidative stress parameters and DNA damage. The mean tail length of two exposure groups was significantly higher as compared to control. These findings showed that during long-term chromium exposure, chromium is absorbed in the body, which may be distributed in the various tissues and organs of exposed workers. The present study revealed that occupational exposure to hexavalent chromium can lead to oxidative stress and DNA damage in electroplating workers. DNA damage and blood chromium level may serve as an efficient biomarker in tannery workers exposed to hexavalent chromium.

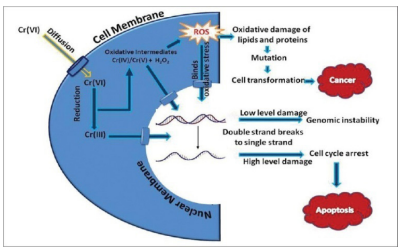

Figure 1.6 Reduction of Cr (VI) and DNA damage inside cell

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