

Abstract

Glucose/xylose isomerase (E.G. 5.3.1.5) (GXI) catalyzes the conversion of D-xylose and D-glucose to D-xylulose and D-fructose respectively. The enzyme plays an important role in the industrial production of high fructose corn syrup (HFCS) which is used as a sweetener in the food industry. Interconversion of xylose to xylulose by GXI serves a nutritional requirement in saprophytic bacteria and has a potential application in the bioconversion of xylan-containing agricultural biomass to ethanol (Parrish, 1970; Katz et al, 1972). *Streptomyces* species are aerobic, Gram positive saprophytic bacteria which typically colonize habitats such as soil. Commercial production of organic acids, antibiotics and industrially important enzymes such as glucose isomerase, xylanase, protease etc. by *Streptomyces* fermentation and the potential for improving the economics of these processes have stimulated interest in the application of novel genetic techniques to *Streptomyces* species. Recombinant DNA technology offers a means to isolate and manipulate the GXI gene to alter the thermostability and substrate specificity to suit its application in HFCS production. The present studies deal with some of the molecular and biochemical aspects of the glucose/xylose isomerase from *Streptomyces* sp. NCIM 2730, an isolate from the National Chemical Laboratory, Pune, India.

The main features of the work are as follows :

1. Molecular cloning, expression and sequence analysis of glucose/xylose isomerase gene 2. Synthesis of recombinant glucose / xylose isomerase : Effect of coordinated addition of specific amino acids 3. Characterization of acid-induced unfolding intermediates of glucose / xylose isomerase 1. Molecular cloning, expression and sequence analysis of Glucose/xylose isomerase A partial genomic library of *Streptomyces* sp. NCIM 2730, constructed in pUC8 vector was screened for the presence of GXI gene using an 18-mer mixed oligonucleotide probe (5'-GTCACGTTCCACGACGAC3') complementary to a highly conserved six-amino acid sequence of GXI from Actinomycetes. Eight clones which hybridized with the radiolabelled oligoprobe showed the ability to complement xylose isomerase-defective *E.coli* mutants. The restriction map of the insert from one (pMSG27) of the eight GXI-positive clones showing detectable GXI activity, was constructed. The cloned gene has unique restriction sites for Bgl II and two restriction sites for Pvu II, EcoR I and Sma I. GXI-deficient strains of *E.coli* were able to utilize xylose as the sole carbon source for their growth upon transformation with pMSG27. *E. coli* JM105 (pMSG27) and *E. coli* JC1553 (pMSG27) were inducible by IPTG suggesting that the expression of the cloned gene was under the control of the lac Z promoter. Western blot analysis revealed that, the cloned gene was expressed as a fusion protein of Mr 110. The present chapter deals with the screening of genomic library, restriction mapping of the GXI encoding DNA fragment, characterization of the recombinant gene product and DNA sequence analysis. The partial sequence of the 1.1 kb insert revealed a 50% homology with reported GXIs from bacterial sources.
2. Synthesis of recombinant Glucose/xylose isomerase : Effect of coordinated addition of amino acids The amplified expression of a recombinant protein is known to lead to an intracellular depletion of specific amino acid pools which in turn may affect the production of the desired protein. In order to counteract and overcome such a situation during the fermentation of the recombinant *E.coli* JM109 (pMSG27) containing the glucose/xylose isomerase (GXI) gene from *Streptomyces* sp. NCIM 2730, the effect of addition of different amino acids on the specific activity of GXI was studied. The amino acid composition of GXI from *Streptomyces* sp. NCIM 2730 reveals predominance of aspartic acid, glutamic acid and glycine. Therefore, the effect of coordinated addition of the assorted combinations of these three amino acids on the synthesis of

recombinant GXI was studied and the results were analyzed using a factorial design. The following conclusions were derived from the analysis of two factor interactions of the three amino acids (a) The interaction between the aspartic and glutamic acid is independent of aspartic acid concentration but is affected by the increasing concentrations of glutamic acid, (b) The effect of aspartic acid concentration is more than that of glycine and (c) During the interaction of glutamic acid and glycine, the effect of glutamic acid is more prominent than that of glycine. The three factor interaction analyses reveal that the effect of the three amino acids is in the order aspartic acid > glutamic acid > glycine.

3. Characterization of acid-induced unfolding intermediates of Glucose/xylose isomerase.

Previous studies that have contributed towards our understanding of the protein folding problem have mainly dealt with monomeric proteins. Similar studies on oligomeric proteins are few and provide an insight regarding the tertiary and quaternary interactions. Acid-induced unfolding of the tetrameric glucose/xylose isomerase from *Streptomyces* sp. NCIM 2730 has been investigated using fluorescence quenching, second derivative spectroscopy, hydrophobic dye-binding (ANS) and circular dichroism techniques. It has been shown that GXI undergoes a two-step unfolding transition when the pH is lowered from 7.5 to 2. Based on its increased hydrophobic exposure, increased accessibility to quenching by acrylamide and native-like secondary structure, the intermediate at pH 3.2 was defined as a molten globule state of GXI. The second intermediate at pH 5.0 is suggested to be an intermediate on the N->MG pathway, characterized by its pronounced secondary structure and ANS binding.