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PURIFICATION AND CHARACTERISATION OF GLUCOSE (XYLOSE) ISOMERASE FROM CHAINIA SP. (NCL 82-5-1)*

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Glucose (xylose) isomerase is an important enzyme in high fructose syrup industry. The enzyme generally occurs intracellularly and is specific for both glucose and xylose. A rare actinomycete **Chainia** sp. (NCL 82-5-1) produces extracellular specific glucose and xylose isomerases and an intracellular glucose (xylose) isomerase (4). The intracellular enzyme is isolated by cell autolysis and purified by preparative polyacrylamide gel electrophoresis. It's properties are studied and compared with those of extracellular specific xylose isomerase (5). The intracellular enzyme has a molecular weight of 1,58,000 daltons with four equal subunits of 40,700 daltons. The N-terminal amino acid sequence analysis shows Arg at the N-terminal. Diethylpyrocarbonate inhibited the enzyme and the inhibition kinetics study shows the presence of atleast 2 essential His residues. The amino acid analysis shows the absence of Cys and a high proportion of hydrophobic and acidic amino acids.

Glucose (xylose) isomerase or more appropriately xylose (glucose) isomerase (EC 5.3.1.5) has a physiological function of converting D-xylose to D-xylulose, the first step in catabolism of xylose. This enzyme also produces D-fructose from D-glucose which allows its commercial use in producing sweet syrups from starch hydrosylate and has the largest market as an immobilised enzyme (1). The enzyme has been obtained mainly from Streptomyces, bacteria , yeasts and yeast-like organisms. Mostly the enzyme occurs intra-

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cellularly except for reports on S. glaucescens (2) and S. olivaceous strain 13 (3). An interesting report on an actinomycete, Chainia sp., appeared in 1983 which describes the production of two separate extracellular substrate specific D-glucose and D-xylose isomerases along with an intracellular D-glucose (xylose) isomerase (4). This created curiosity about the origin of these three enzymes which can be resolved only after their detailed enzymatic and structural studies. Among these extracellular D-xylose isomerase has been purified and characterise (5). We report here purification and characterisation of the intracellular D-glucose (xylose) isomerase.

MATERIALS AND METHODS

Chainia sp. culture was grown for 96 h on a rotary shaker at 30°C in modified Weber's medium (2), prepared in distilled water and was devoid of $CaCO_3$. The cells were harvested by centrifugation (7000 rpm x 15 min), washed, suspended in 0.05 M sodium phosphate buffer pH 7.5 (**I**), frozen and thawed thrice and incubated at 60°C for 2 h with the addtion of 2% toluene. The debris was separated by filtration. Hereafter unless otherwise stated, all the operations were carried out at 0-5°C. The cell free extract was concentrated by $(NH_4)_2S$ precipitation (90%) saturation). The precipitate was dissolved and dialysed against **I**. The enzyme obtained was loaded on preparative polyacrylamide rod gel (5 x 12 cm) electrophoresis (prep-PAGE) at pH 7.5 which was carried out by the method of Davis et al. (6). After the completion of electrophoresis, a thin strip of the rod gel was cut and enzyme was stained by modifying the procedure of Yamanaka (7) where the gel strip was incubated in the reaction mixture at 70°C for 2 min so that the enzyme could be located within 3 min. The corresponding portion of the main gel was cut and extracted with 0.25 M of I containing 0.005 M $MgSO_4.7H_2O$ (**II**). The extract was concentrated and dialysed against 0.05 M of **II**. Acrylamide impurities were removed using batchwise DEAE-cellulose chromatography (adsorption at 0.01 M, II and elution with 0.25 M, II) and gel filtration (Sephadex G-200 column (1.7 x 110 cm) using 0.05 M, **II**).

For molecular weight determination the gel filtration method of Andrews (8) and SDS polyacrylamide gel electrophoresis (SDS-PAGE) method of Weber and Osborn (9) was adopted. The isoelectric point (pI) was determined using "A device for miniscale isoelectric focusing of proteins" (10). Partial N-terminal sequence analysis was carried out using the method of Chang (11). The amino acid composition was analysed by the method of Spackman et al. (12); Moor (13) and Goodwin and Morton (14). Thermostability was determined at pH 7.5 for 10 min at different temperatures and pH stability at various pHs was tested at 65°C for 10 min. Activation energy was calculated using Arrhenius plot. Action of the enzyme on different sugars was studied using D-glucose, D-mannose, D-galactose, α-methyl-D-glucoside, L-rhamnose, D-glucose-6-phosphate, D-xylose, D-ribose and L-arabinose (final concentration in the reaction mixture was 0.1 M for hexoses and 0.005 M for pentoses). Ketohexoses and ketopentoses were assayed as described earlier (15). In order to study the metal ion requirement the enzyme was treated with EDTA and incubated with various metal ions at 37°C for 30 min and then assayed for enzyme activity. Different enzyme inhibitors tested were Tris, barbitone, borate, xylitol, sorbitol,

dulcitol, mannitol, Cu^{2+}, Ca^{2+}, Ba^{2+}, KCl, Fe^{2+}, Fe^{3+}, Sn^{2+}, Zn^{2+}, Mn^{2+}, SDS, Tween-20, Tween-80, cetyltrimethylammonium bromide (CTAB), urea Triton X-100, sodium deoxycholate and NP-40.

For the active site amino acid modification, various reagents used were parahydroxymercuribenzoate, iodoacetate, $HgCl_2$ and cysteine for -SH group; carbodimide (EDAC) for COO^- group; N-acetylimidazole for tyrosine; N-bromosuccinamide for trytophan; phenyl methanesulphonyl fluoride for serine; acetylation for NH_2 group; 2,3 butanedione for arginine; trinitrobenzene sulphonate for lysine and diethylpyrocarbonate (DEP) for histidine (16). Inhibition kinetics of DEP was studied according to Levy et al (17). Assay for glucose isomerase and xylose isomerase were carried out as described earlier (15). Resultant glucose in fructose isomerase assay was estimated by the method of Bergmeyer (18).

RESULTS AND DISCUSSION

The purification of glucose (xylose) isomerase is summarised in Table 1. Purity of the enzymic preparation was checked by analytical-PAGE, pH 7.5 at each step of purification. Fig.1 shows the protein pattern before and after prep-PAGE. The enzyme preparation was homogeneous on PAGE at pH 8.9, 7.5 and SDS-PAGE (Fig.1) and isoelectric focusing. Enzyme was stable in presence of 5 mM Mg^{2+}. Molecular weight by gel filtration was 1,58,000 daltons and SDS-PAGE revealed the presence of 4 subunits of equal molecular weight (40,700 daltons

Table 1. Summary of purification of glucose (xylose) isomerase from *Chainia* sp.

Step No.	Step	Volume ml	Total activity Units	Total protein mg	Specific activity Units/mg protein	Purification fold	Yield %
	Crude extract	100	7.1	205	0.035	1	100
I	Ammonium sulphate precipitation	10	6.8	175	0.039	1.1	96
II	Prep.PAGE eluate dialysed and lyophilised	1	5.4	0.668	8.08	231	76
III	DEAE-cellulose chromatography	1	3.3	0.407	8.11	232	46
(Enzyme from 10 batches of Step III)							
IV	Gel filtration on Sephadex G-200	0.1ml (x 10)	3.0 (x 10)	0.369 (x 10)	8.13	233	42

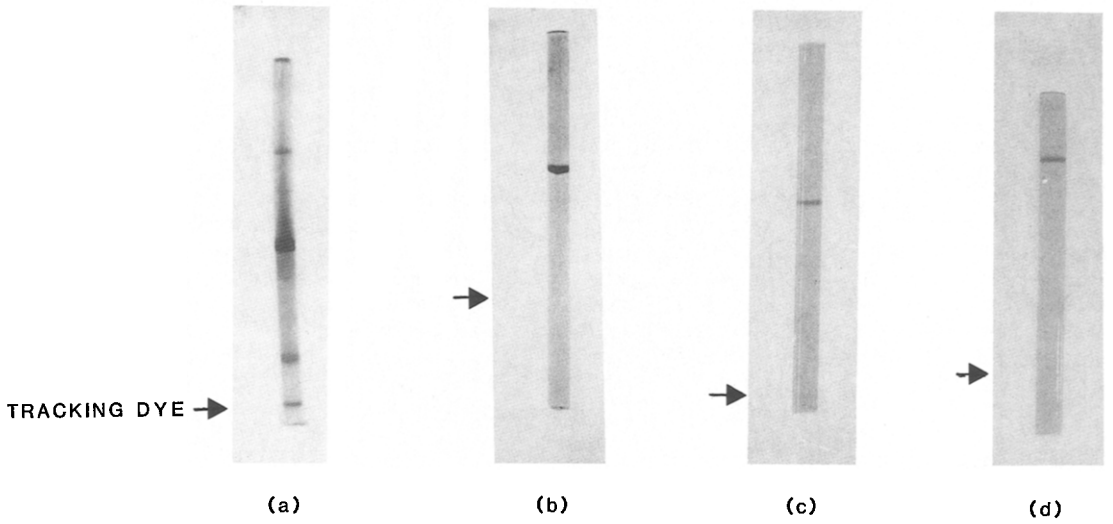

Fig. 1. PAGE patterns of glucose (xylose) isomerase from **Chainia** sp.

(a) Ammonium sulphate precipitated and dialysed form at pH 8.9.
(b) Purified enzyme protein at pH 8.9.
(c) Purified enzyme protein at pH 7.5.
(d) SDS-PAGE pattern.

each) which is similar to the enzyme from S. albus YT-5 (19). pI of the enzyme was found to be 4.0 which shows its acidic nature. The sequence of N-terminal 9 amino acids was NH_2-Arg-His-Ala-Gly-Ser-Ala-His-Thr-Phe---. Other reports on sequence analysis of glucose (xylose) isomerase are by Hogue-Angeletti (20) and Kasumi et al (21). It is worth nothing that there is no homology between the determined N-terminal sequences of these three enzymes. Amino acid analysis of the **Chainia** enzyme (Table 2) shows the predominance of hydrophobic and acidic residues and absence of Cys, similar to other reports (22

Optimum temperature and pH for the enzyme was 75°C and 7.5, respectively. The activation energy was 50.55 Kcal $mole^{-1}$ of enzyme. The enzyme retained 97% of its activity when incubated at pH 7.5 and 65°C for 10 min. The optimum temperature, optimum pH, pH stability and thermostability of the enzyme indicates its industrial potential when compared to other enzymes described (23).

Among various substrates tested only D-glucose and D-xylose were isomerised by the enzyme which is also a common characteristic of the other reported glucose (xylose) isomerases (23). K_m for D-glucose and D-fructose was 0.59 M and for D-xylose was 0.008 M which indicates that this enzyme, like almost all other glucose (xylose) isomerases

Table 2. Amino acid composition of glucose (xylose) isomerase from Chainia sp.

Amino acid	Residues per mole
Lysine	67
Histidine	21
Arginine	20
Aspartic acid + Asparagine	107
Threonine	89
Serine	107
Glutamic acid + Glutamine	161
Proline	147
Glycine	228
Alanine	129
Half Cystine	-
Valine	123
Methionine	12
Isoleucine	11
Leucine	69
Phenylalanine	30
Tyrosine	27
Tryptophan	16
Total :	1364

is more appropriately a D-xylose (glucose) isomerase. The enzyme lost its activity when treated with EDTA. Mg^{2+} and Co^{2+}, individually could restore the activity upto 34% and 27%, respectively, whereas Mg^{2+} and Co^{2+} together (0.002 M) could restore the total enzyme activity. Following are the potent inhibitors of the enzyme: Tris (0.1 M, 55%), xylitol (0.12 M, 96%), sorbitol (0.2 M, 80%), Cu^{2+} (0.005 M, 100%), Ca^{2+} (0.005 M, 60%), CTAB (0.1%, 85%) and Triton X-100 (0.1%, 46%). Thus alomost all known inhibitors of glucose isomerase also inhibit the enzyme from Chainia sp. The effect of detergents on the enzyme activity is reported for the first time here. Among various chemical modifications reagents only DEP at 1.25 mM inhibited the enzyme activity by 77%. The kinetic studies and plots of the logarithm of pseudo-

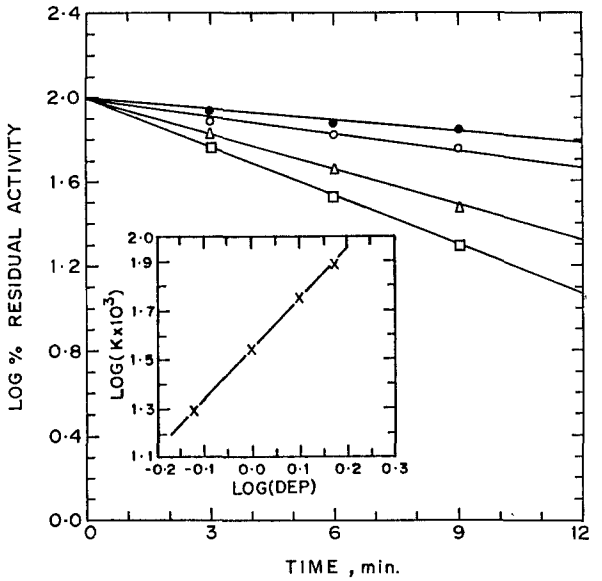

Fig. 2. Inactivation of glucose (xylose) isomerase by DEP.

The enzyme (320 µg in 1.2 ml) was incubated with different amounts of the reagent (0.75, 1.0, 1.25 and 1.5 mM) in 0.05 M Sodium phosphate buffer, pH 7.0 at 25°C. Samples were withdrawn at various time intervals and estimated for residual activity.

Inset, plot of log of pseudo-first order inhibition rate constant (k) against log of DEP concentration.

first order rate constants against the logarithm of the corresponding DEP concentration resulted in a straight line with a slope of 2 (Fig.2), indicating the presence of atleast two essential His residues on every active unit of the enzyme. The pKa value of the active group observed from plot of K_m vs pH was 7.1 which is close to the pKa value of imidazole group of histidine. The inhibition by DEP could be reversed by $NH_4OH.HCl$ (0.2 M) to 25% from the residual 7% activity. All these results provide an evidence for histidine being present at the active site of this enzyme. Very recently Vangrysperre *et al.* (24) have demonstrated His at the active site of the enzyme from 4 different sources. Other reports predicted the presence of a sulfhydryl group at the active site of the enzyme from *Streptomyces* sp. YT-5 (19) and *Pasteurella pestis* (25).

The extracellular D-xylose isomerase from **Chainia** sp. is a single polypeptide chain with molecular weight 71,000 having Arg and His as the active site groups (5) whereas the intracellular glucose (xylose) isomerase consists of 4 subunits with a total molecular weight of

1,58,000 and only His at the active site. Thus the possibility of different origins of these two enzymes cannot be ignored.

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