**EFFECT OF CALCIUM AND XYLOOLIGOSACCHARIDES ON XILOSE ISOMERASE ACTIVITY FOR ISOMERIZATION OF XYLOSE TO XYLULOSE**

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**Highlights**

* Kinetic parameters of the enzymatic xylose-to-xylulose isomerization were estimated.
* Ca2+ ionsare competitive inhibitors of the isomerization enzymatic reaction.
* Xylobiose inhibition is only important at high concentrations.

**1. Introduction**

Xylose isomerase (XI) catalyzes the reversible isomerization of xylose to xylulose and of glucose to fructose. Its molecular mass is around 173 kDa, formed by four identical subunits of approximately 47 kDa each. Bivalent ions (Mg2+, Mn2+ and Co2+) act as important cofactors of the enzyme, some of them being essential components in the reaction medium in order to keep the isomerase activity. XI is one of the enzymes with the highest market demand in the food industry, used for the production of high-fructose corn syrup (HFCS) through the isomerization of glucose to fructose. Due to this industrial application of XI, the kinetics of this reaction was well investigated.

On the other hand, there are few reports in the literature about the enzymatic xylose-xylulose isomerization, most of them between the years 1980-1990, and related to the application of the reaction in the simultaneous isomerization and fermentation of xylose (Simultaneous Isomerization and Fermentation, SIF). These studies aimed at the production of 2G ethanol from hemicellulose, through fermentation of xylulose by *Sacharomyces cerevisiae*, the microorganism conventionally used in the 1G ethanol production. However, the genetic modification of *S. cerevisiae* to allow *in-vivo* isomerization in order to achieve the direct fermentation of xylose was almost the only studied route in the following 20 years. This approach is still not implemented in the industry, so the SIF process study was resumed [1]. Furthermore, XI was also used as a component in a biocatalyst for ethanol production from xylo-oligomers, the Simultaneous Hydrolysis, Isomerization and Fermentation process (SHIF) [2]. In the SHIF process, xylanases hydrolyze xylo-oligomers to xylose, which is then isomerized to xylulose by XI, and xylulose is converted to ethanol by non-modified *S. cerevisiae*. The biocatalyst also contains calcium carbonate in order to control the intra-particle pH. Xylobiose and Ca2+ ions are potential inhibitors of the isomerization of xylose to xylulose in the SHIF process. Within this context, the kinetics of this reaction is investigated here.

**2. Methods**

2.1 Xilose isomerase activity assay: initial velocity of xylulose formation is determined using 5 mL of substrate solution (2 mol.L-1 xylose in 50 mmol.L-1 tris-maleate buffer pH 7.0 containing 50 mmol.L-1 MgCl2.6H2O and 2.5 mmol.L-1 CoCl2.6H2O), at 60 °C. Xylulose was quantified using the cysteine-carbazol method [3].

2.2 Preparation of xylobiose rich solution according to [4]. Beechwood xylan solution (50 g.L-1 in 50 mM sodium citrate buffer pH 5.5) was hydrolyzed by soluble xylanase NS22036. Xylo-oligosaccharides concentration was determined via HPLC using a SugarPak I column (Waters, Milford, USA).

2.3 Influence of substrate, Ca2+ ions and xylobiose (X2) on the rate of reaction at 35 °C: concentrations were varied, for xylose (15-300 g.L-1), Ca2+ ions (0-16 g.L-1) and X2 (0 - 9.1 g.L-1).

**3. Results and discussion**

A Michaelis-Menten model with competitive inhibition represented well the influence of Ca2+..The kinetic model describes the competition between Ca2+ and Mg2+ ions for the metal site within the active site of XI.

Isomerization rates were slightly affected by the presence of X2 at substrate concentration of 45 g.L-1. Inhibitory effects were better perceived at higher substrate concentrations, keeping the amount of X2 fixed. A Michaelis-Menten rate expression with uncompetitive inhibition fitted well to the experimental data.

**4. Conclusions**

This work showed that Ca2+ competes with Mg2+ for the metal site in the enzyme structure. X2 is a uncompetitive inhibitor of the reaction, affecting the reaction rates more severely at higher substrate concentrations.

**References**

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