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SUBSTRATE SPECIFICITY OF YEAST INVERTASE IN TRANSGLYCOSYLATION REACTIONS

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The substrate specificity of purified yeast invertase isolated from Saccharomyces cerevisiae *in transglycosylation reactions was determined. The enzyme is specific for primary alcohols. The yeast activity is a function of the alkyl length and substrate hydrophobicity (*n*-butyl, isobutyl, isoamyl alcohols).*

Key words: *Saccharomyces cerevisiae*, yeast invertase, active enzyme.

The enzyme invertase (β-fructofuranoxidase, K.F. 3.2.1.26) is known to catalyze the cleavage of saccharoses into glucose and fructose. The enzyme can catalyze transferase reactions in addition to hydrolytic ones. In the former, the substrate (fructose) undergoes substitution of certain groups by alcohol alkyls [1, 2]. This enzyme is especially active in yeast. Therefore, it represents a potential source for producing purified enzyme preparations. One of the most important features of enzymes is the substrate specificity. Few reports of the specificity of invertase for transalkylation have appeared. Therefore, we attempted to determine the specificity of *Saccharomyces cerevisiae* invertase for various substrates (with linear and branched structures) and various chemical bonds in transglycosylation reactions.

The results showed that the enzyme is more active for alcohols with 1-5 C atoms and the hydroxyl in the α-position. A substrate (alcohol) structure in which the OH is bonded to the first C atom also produces high catalytic activity. The invertase activity depends not only on the substrate groups, but also on the nature of their position in the C chain. Thus, the synthesis of isopropylfructoside can be used to judge the effect of a methyl in the α-position (secondary alcohol) on invertase activity. In other words, isopropylfructoside was not synthesized in our experiments if the secondary alcohol had an α-methyl.

The mechanism of invertase activity on alkyl substrates is not well studied. The reduced catalytic activity in instances where the substrate is a branched alcohol is apparently due to steric hindrance toward access of a branched alcohol to the enzyme catalytic center. Isopropyl alcohol (model), in which the reactive OH is located on a secondary C atom, provides an example. It should be mentioned that invertase activity increased dramatically if the secondary C atom was far removed from the reactive OH. Alcohols (isobutyl and isoamyl) in which the secondary C atom is one (isobutyl) or two (isoamyl) C atoms removed from the OH provide examples of this.

The invertase specific activity for methanol is 16.216 AU/mg. This is greater than the activity for ethanol by 1.14 times (14.216 AU/mg); for propyl, 1.87 (8.666 AU/mg), for isobutyl, 1.75 (9.250 AU/mg), for *n*-butyl, 1.44 (11.293 AU/mg), and for isoamyl, 1.02 (15.873 AU/mg). Therefore, it is hypothesized that the main criteria for substrate recognition by invertase is the placement of the OH on the first C atom, the branching, and the proximity of the secondary C atoms to the reactive OH of alcohol.

The Michaelis constant for invertase under optimal conditions (Fig. 1) is 10 mM substrate, which in our work was isoamyl alcohol.

The results also reveal that the enzyme activity and the solubility of various substrates are definitely related. In particular, it was demonstrated that the specific activity tends to increase with decreasing solubility of the alcohols in water (Fig. 2).

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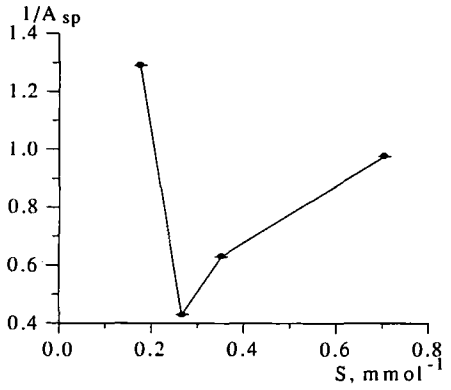

Fig. 1.

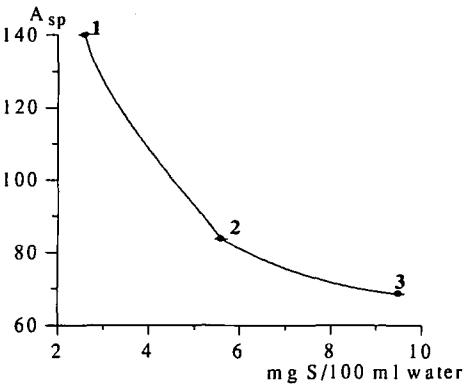

Fig. 2

Fig. 1. Invertase activity as a function of substrate concentration: ordinate, inverse invertase specific transferase activity; abscissa, inverse substrate concentration (isoamyl alcohol, 1 mmol).

Fig. 2. Invertase activity as a function of substrate solubilities (alcohols) in water: ordinate, specific transferase activity of invertase; abscissa, solubility of various alcohols in water (mg/100 ml water). Isoamyl (1), isobutyl (2), and butyl (3) alcohols.

Thus, *Saccharomyces cerevisiae* invertase, which is specific for a wide range of substrates, is most active for alcohols with a primary OH. Criteria for recognizing the substate are the placement of the OH on the first C atom, the branching of the C chain, and the proximity of secondary C atom to the OH. The hydrophobicity of the alcohol is just as important. Analogous results were obtained in experiments with several other hydrolases (acylase, lipase, protease, phospholipase) [3].

EXPERIMENTAL

We used purified invertase (K.F. 3.2.1.26) isolated from Baker's yeast *Saccharomyces cerevisiae* [4]. The invertase activity was determined as follows. A solution containing 4% saccharose and 20% (by vol.) CH_3CN was divided into two equal portions. One of these was treated with 50 μl (0.65 mg) of enzyme solution; the other (control), with the same amount of enzyme inactivated by boiling. The transferase activity of invertase was found by the decrease in the amount of isoamyl alcohol, which was determined by GC on a Chrom-5 chromatograph [5]. The transferase activity of invertase was calculated by the literature method [4]. The activity unit of transferase was taken as the amount of isoamyl (or other) alcohol (mM) bound to alkylfructoside in 1 h at 25°C and pH 4.5.

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