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α,β-dicarbonyl reduction by *Saccharomyces* D-arabinose dehydrogenase

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Abstract

An α,β-dicarbonyl reductase activity was purified from *Saccharomyces cerevisiae* and identified as the cytosolic enzyme D-Arabinose dehydrogenase (*ARA1*) by MALDI-TOF/TOF. Size exclusion chromatography analysis of recombinant Ara1p revealed that this protein formed a homodimer. Ara1p catalyzed the reduction of the reactive α,β-dicarbonyl compounds methylglyoxal, diacetyl, and pentanedione in a NADPH dependant manner. Ara1p had apparent K_m values of ~14 mM, 7 mM and 4 mM for methylglyoxal, diacetyl and pentanedione respectively, with corresponding turnover rates of 4.4, 6.9 and 5.9 s^{-1} at pH 7.0. pH profiling showed that Ara1p had a pH optimum of 4.5 for the diacetyl reduction reaction. Ara1p also catalyzed the $NADP^+$ dependant oxidation of acetoin; however this back reaction only occurred at alkaline pH values. That Ara1p was important for degradation of α,β-dicarbonyl substrates was further supported by the observation that *ara1*-Δ knockout yeast mutants exhibited a decreased growth rate phenotype in media containing diacetyl.

Keywords: D-Arabinose; *ARA1*; Dehydrogenase; Dicarbonyl; Diacetyl; ARA1p

1. Introduction

Metabolic processes in the yeast *Saccharomyces cerevisiae* produce a variety of intermediate by-products that may be toxic or mutagenic to the cell. For example, biosynthesis of the branched amino acids isoleucine, leucine, valine via the regulated ILV pathway [1] can lead to the accumulation of the precursor metabolite α-acetohydroxy butyrate and α-acetolactate. Under oxidative conditions these compounds can undergo non-enzymatic decarboxylation [2,3] to produce 2,3-pentanedione and diacetyl (2,3-butanedione) (Fig. 1A). The toxic metabolite methylglyoxal can also be generated as a by-product of non-enzymatic degradation of triose phosphates stemming from glycolysis (Fig. 1A) [4]. α,β-dicarbonyls such as diacetyl and methylglyoxal are highly reactive compounds that can react with arginine, lysine, and cysteine residues resulting in damage and cross linking of proteins [5]. In mammalian cells methylglyoxal and diacetyl have been implicated in a number of diseases that include diabetic vascular complications [6], neurodegenerative diseases [7], atherosclerosis, and general cellular deterioration and aging [8]. Several proteins have been shown to be susceptible to chemical modification, including ovalbumin [9], plasminogen [10], and glyceraldehyde phosphate dehydrogenase [11]. Removal of these dicarbonyl compounds is therefore essential for maintenance of cellular health. While *Saccharomyces cerevisiae* displays a regulatory mechanism when exposed to methylglyoxal [12], little is known about the cellular responses to diacetyl produced during amino acid metabolism.

Aside from being an excellent model system for eukaryotic cell studies, *Saccharomyces* spp. have significant commercial application in alcoholic beverage production. During the fermentation process yeast release the metabolite diacetyl, an undesirable compound that imparts a butterscotch-like aroma and unpleasant flavour that can be detected down to ~1 μM concentrations [13]. The presence of diacetyl in beer requires a lengthy maturation period during which yeast enzymatically reduce diacetyl to acetoin (2-hydroxy-3-butanone), a more flavour-neutral compound with a significantly increased aroma threshold (Fig. 1B).

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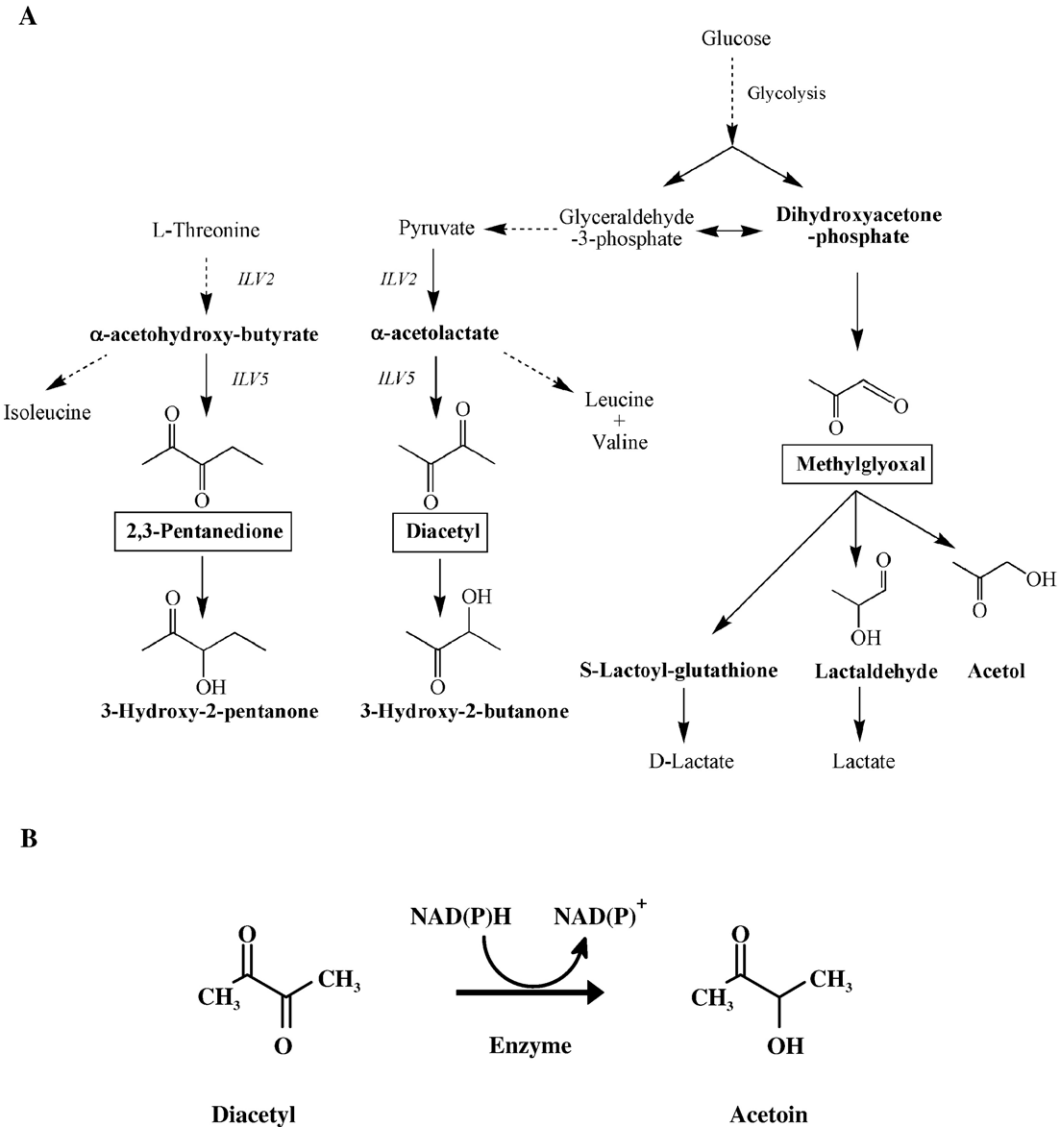

Fig. 1. Viccinal dicarbonyl production. (A) Schematic of metabolic pathways responsible for the generation of the α,β dicarbonyl compounds diacetyl, pentanedione, and methylglyoxal. *ILV2* and *ILV5* correspond to the enzymes α-acetolactate synthase and α-acetohydroxybutyrate synthase respectively. (B) Reaction scheme showing NAD(P)H mediated catalysis reduction and oxidation of diacetyl to acetoin (3-hydroxy-2-butanone).

Despite the physiological and industrial significance of this compound, little is known about the yeast enzymes involved in reduction. Historically, yeast alcohol dehydrogenase was shown to catalyze diacetyl reduction [14], but the high Michaelis constant (K_m) of 250 mM suggests that this enzyme would exhibit an extremely slow catalytic efficiency since diacetyl concentrations seldom exceed 100 μM under physiological concentrations. Heidlas and Tressl [15] purified two enzymes of 36 and 75 kDa capable of catalyzing diacetyl reduction in *S. cerevisiae* with a K_m of 2 and 2.3 mM respectively for diacetyl. Both enzymes also catalyzed the reduction of 2,3-pentanedione and displayed K_m values of 2 and 1.5 mM, respectively. However the sequences of these proteins were not determined. Ypr1p, a 36.4 kDa aldo-keto reductase from bakers yeast, was shown to catalyze diacetyl reduction and yielded a K_m of 5.7 mM [16]. Partially purified diacetyl reductase activities from ale (*S. cerevisiae*) and lager (*S. pastorianus*) yeast displayed K_m values 79 mM and 15 mM for diacetyl respectively [17]. More recently a diacetyl reductase activity purified from anaerobic *S. cerevisiae* cultures was shown to be Old Yellow enzyme (OYE). Kinetic characterization of the Oye2p isoform shows that this enzyme reduced diacetyl and pentanedione with biphasic kinetics (van Bergen et al. submitted for publication). K_m values of ~2 and ~180 μM for diacetyl and pentanedione were determined for Oye2p.

In this study we partially purified a diacetyl reductase activity from *S. cerevisiae* grown aerobically. Mass spectrometry analysis identified this protein as D-arabinose dehydrogenase (Ara1p). The enzymatic activity of this enzyme was validated by over expression and kinetic analysis of recombinant Ara1p with the substrates methylglyoxal, diacetyl and pentanedione.