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MiniReview

Alcohol oxidase: A complex peroxisomal, oligomeric flavoprotein

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Abstract

Alcohol oxidase (AO) is the key enzyme of methanol metabolism in methylotrophic yeast species. It catalyses the first step of methanol catabolism, namely its oxidation to formaldehyde with concomitant production of hydrogen peroxide. In its mature active form, AO is a molecule of high molecular mass (600 kDa) that consists of eight identical subunits, each of which carry one non-covalently bound flavin adenine nucleotide (FAD) molecule as the prosthetic group. In vivo, the protein is compartmentalized into special cell organelles, termed peroxisomes.

AO is an abundant protein and its synthesis is strictly regulated by repression/derepression and induction mechanisms that occur at the transcriptional level. Various aspects of its sorting and assembly/activation render AO a unique protein.

Recent developments of AO synthesis, sorting and assembly/activation are highlighted in this paper.

Keywords: Alcohol oxidase; Protein sorting; Dofactor binding; Peroxisome

1. Introduction

Several yeast species, belonging to the genera *Pichia* and *Candida*, are able to utilize methanol as sole source of carbon and energy. Growth of these organisms on methanol requires the function of specific organelles, called peroxisomes, that contain the key enzymes of methanol metabolism. The first enzyme of methanol utilization is alcohol oxidase (AO; EC 1.1.3.13) that belongs to the family of glucose-methanol-choline (GMC) oxidoreductases [1]. The generalized name AO is adopted from the fact that in vitro the enzyme also oxidizes other short aliphatic alcohols [2]. AO protein and the regulation of the gene encoding AO (*AOX*) have been studied for decades for several reasons. Firstly, the promoter that controls *AOX* expression is known to be one of the strongest promoters in nature and, at the same time, one of most tightly controlled yeast promoters. These features render methylotrophic yeast species highly attractive hosts for heterologous gene expression [3–6]. Secondly, AO protein itself is of high industrial interest. It constitutes, for instance, the main component of an alcohol sensor [7–9] because of various favourable properties such as: (i) a high affinity for primary alcohols (μM range); (ii) the high stability of its active form; and (iii) its easy availability, as AO protein may comprise up to 30% of total cellular protein [10–12].

2. The *AOX* gene

Several genes encoding AO protein have been cloned to date from various organisms (Table 1), in particular

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Table 1
Genes encoding AO protein

Organism	Gene designation	Remarks	References
Methylotrophic yeast			
Hansenula polymorpha (*Pichia angusta*)	*MOX*		[85]
Pichia pastoris	*AOX1, AOX2*		[24]
Pichia methanolica	*AUG1 (MOD1), AUG2 (MOD2)*		[23,86]
Pichia pinus	*AOX1*		Genbank, GI:37694459; unpublished
Candida boidinii	*AOD1*		[87]
Filamentous fungi			
Penicillium chrysogenum	*AOX*, induced under conditions of penicillin production		[88]
Cladosporium fulvum	*AOX1*, induced by carbon starvation and during later stages of infection; deletion results in reduced pathogenicity		[89]
Helminthosporium (*Cochliobolus*) *victoriae*	–	Hv-p68; RNA-binding protein	[90]
Aspergillus nidulans	–	Hypothetical protein with high homology to AO from methylotrophic yeast	Genbank, GI:40747510; unpublished

from yeasts and filamentous fungi. Although AO proteins from filamentous fungi show a high degree of homology (65–70% identity) to those of methylotrophic yeast species, their function is not related to methanol metabolism as in yeasts. Various acronyms are used to indicate genes encoding AO (Table 1). For simplicity all genes identified thus far are designated *AOX* throughout this review.

3. *AOX* gene expression

The *Hansenula polymorpha AOX* gene [13–15] contains a large promoter region of approximately 1.5 kb that is tightly regulated. Three regulatory sequences have been identified within the *AOX* promoter, namely two upstream activation sequences, UAS1 and UAS2, as well as one upstream repressing sequence, URS1. These sequences were observed to bind regulatory proteins, named MBF1, MBF2 and MBF3 (*MOX* Binding Factor), respectively. However, these proteins have not been analysed further [13].

Expression of *AOX* is subject to strong carbon catabolite repression [16,17]. Under such conditions, the *AOX* promoter sequence (P_{AOX}) is organized in nucleosome structures [14], thus being unavailable for the transcription machinery. Prior to initiation of AO synthesis at derepressing or inducing conditions (for instance in the presence of glycerol or methanol), the gene needs to be liberated from nucleosomes via the function of the chromatin remodelling complex. Recently, we showed that HpSwi1p and HpSnf2p, proteins that are homologous to subunits of the SWI/SNF chromatin remodelling complex from *Saccharomyces cerevisiae*, may be involved in this process [18]. The ultimate level of *AOX* expression strongly depends on the cultivation conditions [16]. Also, differences exist in the regulation of *AOX* expression in different yeast species. In *H. polymorpha*, which has one *AOX* gene, low levels of AO can be observed at glucose-limiting conditions (e.g., glucose-containing batch cultures at the early stationary growth phase or in carbon-limited chemostats). The AO protein levels are enhanced when cells are grown in the presence of dihydroxyacetone or glycerol [16]. By contrast, in *Pichia pastoris* cells grown in presence of glycerol, no mRNA of *AOX* has been detected [19]. For very high AO protein levels, expression of the gene requires induction by methanol and can then increase to up to 30% of total cellular protein [11,12]. At these conditions, cells are loaded with peroxisomes (Fig. 1). Similar high AO levels are observed in glucose-limited chemostat cultures supplemented with choline as sole nitrogen source [20]. Interestingly, maximal induction of AO expression is also obtained in carbon-limited mixed substrate chemostat cultures supplemented with glucose and methanol. Both carbon sources are then completely utilized leading to increased biomass production, compared to single carbon source chemostat cultures [21].

In *P. pastoris* and *P. methanolica*, which contain two genes coding for AO (Table 1), differential expression of both genes is observed, a phenomenon that is strictly dependent on cultivation conditions [19,22,23]. This differential expression of both genes is related to the presence of different regulatory sequences [24].