SYNTHESIS OF CATALASE T UNDER ANAEROBIC CONDITIONS IN A MUTANT OF *SACCHAROMYCES CEREVISIAE*

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1. Introduction

The yeast *Saccharomyces cerevisiae* is capable of growing under aerobic or anaerobic conditions. The formation of some proteins of aerobic yeast cells, particularly of a number of heme proteins, is dependent on the availability of oxygen. Among these proteins are mitochondrial cytochromes [1–3], cytochrome *c* peroxidase [4–7] and catalases [2,8,9]. In some instances, as in the case of cytochrome *c* peroxidase [6,7] and of some subunits of cytochrome *c* oxidase [7,10,11], synthesis of the protein part of these enzymes is possible in the absence of oxygen. In other cases, like the yeast catalases A and T [8,9], anaerobically-grown cells lack the corresponding apoproteins.

Especially in those instances where accumulation or correct assembly of the apoproteins is dependent on oxygen, fairly little is known about the mechanism of the oxygen effect. Information on this subject is likely to come from studies that combine a biochemical with a genetic approach. A procedure for the detection of yeast mutants possessing catalase activity under anaerobic conditions has been developed [12]. This paper presents the first results of a biochemical characterization of such a mutant. Our data show that anaerobically-grown cells of this strain are able to synthesize catalase T, one of the two main catalase proteins of *Saccharomyces cerevisiae*.

Abbreviations: TCA, trichloroacetic acid; PPO, 2,5-diphenol oxazole

2. Materials and methods

Strain AS 13 *rho*$^-$ (α *leu*-1 *ade*-1 *cgr*-1 *cas*-1) was isolated in the Department of Genetics, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, after ultraviolet mutagenesis [13] of strain 26 B (α *leu*-1 *ade*-1 *cgr*-1) [14]. The mutant was detected in a plate test by qualitatively testing the catalase activity of colonies grown under anaerobic conditions [12]. The gene designation *cas* has been given to strains able to synthesize catalase under anaerobic conditions. Strain 26 B *rho*$^-$ was obtained from strain 26 B by ethidium bromide treatment [15]. Strain D 273-10 B (α) (ATCC 24657) is a respiratory competent wild-type strain. The strains were grown anaerobically [16] and aerobically on 0.3% glucose on a semisynthetic medium containing Tween-80 and ergosterol as in [17]. In the case of the auxotrophic strains the medium was supplemented with adenine sulfate (20 μg/ml) and L-leucine (30 μg/ml). Aerobic and anaerobic cultures were grown to early stationary phase. Oxygen adaptation was carried out in 0.3% glucose, 0.04 M phosphate buffer, pH 7.4.

Cells were labelled with L-[^{3}H]leucine (50 Ci/mmol, Amersham). Unlabelled leucine was omitted from the growth medium in such experiments without any effect on the growth characteristics of the culture. Soluble extracts for determination of catalase activity and specific immunoprecipitates were obtained as in [8,9]. Immunoprecipitates were analyzed after dissociation in 2.5% dodecyl sulfate, 10 mM dithiothreitol, 10 mM sodium phosphate, pH 7.0, by dodecyl sulfate gel electrophoresis [18] on 7.5% polyacrylamide gel plates. After electrophoresis, gels were treated with 10% PPO in acetic acid for 2–3 h, rinsed with water overnight and dried. Bands were visualized by fluorography [19], the zones corresponding to catalase T protein were cut out, radioactive protein was solubilized with Protosol (New England Nuclear, FRG), and radioactivity was determined in toluene–Triton X-100 scintillation fluid.

Catalase activity was determined as in [20]. Catalase T and catalase A content was determined by electrophoretic and immunological methods as in [21]. Protein was determined with comparable results by the methods of Lowry et al. [22] and of Bradford [23].

3. Results

Since the plate test used for detecting the mutant had indicated that anaerobically-grown strain AS 13 *rho*$^-$ possessed catalase activity this mutant was examined more carefully after growth in liquid medium. As a control, the corresponding *cgr*-1 parent strain was also tested. Since AS 13 was available only in the *rho*$^-$ state, strain 26 B *rho*$^-$ was used for comparison. Table 1 shows that 26 B *rho*$^-$, like other strains tested previously, has no detectable catalase activity when grown under anaerobic conditions. Less than 1% of the catalase activity of the aerobically-grown strain 26 B *rho*$^-$ would have been detectable under the experimental conditions used. In contrast to the parent strain, AS 13 *rho*$^-$ retains 16% aerobic catalase activity if grown anaerobically. In addition, the catalase activity of the aerobically-grown mutant is much higher than that of the parent strain. Electrophoretic tests [21] and examination with specific antisera showed that both strains contained only

Table 1
Catalase T activities in 26 B *rho*$^-$ and AS 13 *rho*$^-$

Strain	Aerobic (U/mg protein)	Anaerobic
26 B *rho*$^-$	4.9	0
AS 13 *rho*$^-$	81.4	13.2

Strains were grown anaerobically or aerobically on 0.3% glucose as described in section 2

catalase T, but no catalase A activity. This finding is in agreement with the observation made recently that glucose-grown *rho*$^-$-strains contain no active catalase A [24].

The result obtained could mean that AS 13 *rho*$^-$ synthesizes active catalase T under anaerobic conditions. It could, however, also be explained by an ability of the mutant strain to adapt either unusually fast or at lower oxygen concentration. To test the latter possibility the anaerobicity of the fermentor system used was carefully controlled. In the case of wild-type strains, nitrogen containing less than 5 ppm oxygen is sufficient for growing cells showing no detectable catalase activity. In the experiments described here, nitrogen purified with an Oxisorb cartridge (Messer Griesheim, FRG), to an oxygen concentration < 0.1 ppm was used. In addition, our system was tested by measuring the cytochrome *c* oxidase activity of promitochondria of strain D 273-10 B, which was grown and harvested under exactly the same conditions as our mutant strain. Whereas the mutant strain reproducibly had high catalase T activity the cytochrome *c* oxidase activity of D 273-10 B in agreement with [3,16], was repeatedly extremely low. Although these results cannot completely exclude the possibility of an adaptation of the strain caused by very low concentrations of oxygen, they make it quite unlikely.

Since AS 13 *rho*$^-$ can form active catalase T during oxygen adaptation in the presence of cycloheximide (see below), poisoning of anaerobically-grown cells with this inhibitor before harvesting does not exclude the possibility of catalase T formation by fast adaptation. The adaptation kinetics of strains AS 13 *rho*$^-$ and 26 B *rho*$^-$ were therefore examined. These results are summarized in fig.1. Whereas a 1 h lag period was observed with 26 B *rho*$^-$

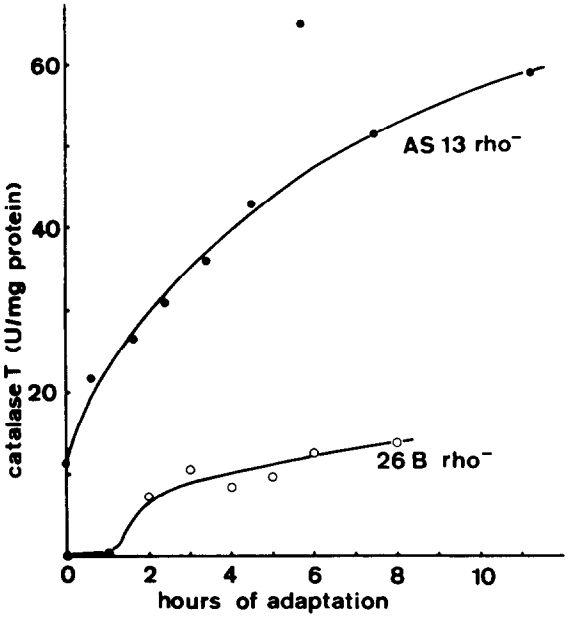

Fig.1. Formation of catalase T during oxygen adaptation Cells grown anaerobically were aerated in 0.3% glucose, 0.04 M phosphate buffer, pH 7.4.

before the appearance of active catalase T, the enzyme activity started to rise immediately in strain AS 13 *rho*⁻. After the lag period the velocities of appearance of active enzyme do not differ dramatically in the two strains. The differences in adaptation kinetics do not seem to be sufficiently great to explain the differences in catalase T content of the anaerobically-grown strains.

Earlier investigations [8,9] have shown that the delay in appearance of active catalase during oxygen adaptation is at least partly caused by the synthesis of enzymatically inactive catalase precursors. The absence of such a lag period in strain AS 13 *rho*⁻ suggested that its anaerobically-grown cells might contain catalase T precursors in addition to active enzyme. As is shown in fig.2, formation of active catalase T during oxygen adaptation in the presence of cycloheximide (50 μg/ml) is possible in AS 13 *rho*⁻, but not in 26 B *rho*⁻. This finding also indicates that the anaerobically-grown mutant contains catalase T precursor molecules.

To determine more rigorously the amount of catalase T protein present in mutant cells grown

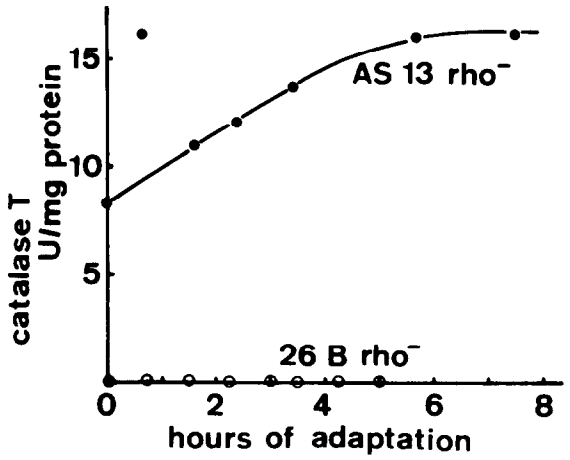

Fig.2. Formation of catalase T during oxygen adaptation in the presence of cycloheximide (50 μg/ml).

anaerobically, labelling experiments were carried out. Catalase T protein labelled with L-[^{3}H]leucine was immunoprecipitated from extracts of cells labelled during anaerobic or aerobic growth, respectively. Radioactivity incorporated into total protein and specific enzyme activities were determined in the same extracts. As can be seen in table 2, the percentage of total protein precipitable with a specific catalase T antiserum is identical for anaerobically- and aerobically-grown cells. On the other hand, the specific catalase activity of extracts from anaerobic cells is considerably lower than that from aerobic cells. If no factors modifying the catalase activity are present in one of the extracts, this would mean that more than half of the catalase T protein present in anaerobically-grown cells is enzymatically inactive, which agrees well with the results of adaptation in the presence of cycloheximide.

It might be noticed that the catalase activity of anaerobically-grown cells given in table 2 is higher than the activities in table 1 and fig.1,2. Control experiments indicate that this difference is caused by slightly different growth conditions used in the labelling experiments and not by a lack of anaerobicity of the cultures.

Similar labelling experiments were carried out with strain 26 B *rho*⁻. No immunoprecipitable catalase T protein could be detected in extracts obtained from anaerobically-grown cells of this strain.

Table 2
Catalase T activity and catalase T protein in AS 13 *rho*$^-$

	Anaerobic	Aerobic
Catalase T activity (U/mg protein)	39.5	86.1
% Acid-precipitable radioactivity recovered in immunoprecipitated apocatalase T	0.11	0.11

Cells were grown anaerobically or aerobically in the presence of 100 μCi and 50 μCi L-[^{3}H]leucine, respectively. Specific catalase T activity and TCA-precipitable radioactivity of soluble extracts was determined. Catalase T protein was precipitated by a specific antiserum, the solubilized immunoprecipitate was purified on 7.5% polyacrylamide gels, radioactive bands were visualized by fluorography, cut out and counted in a liquid scintillation counter

4. Discussion

The results reported here show that we have succeeded in isolating a mutant capable of synthesizing and accumulating catalase T protein under anaerobic conditions in amounts comparable to those present in aerobically-grown cells. The kinetics of oxygen adaptation, adaptation in the presence of cycloheximide and the reduced specific enzyme activity of the catalase T protein from anaerobically-grown cells, indicate that a part of the catalase T protein of cells grown in the absence of oxygen is present as a precursor. Other possibilities to explain the rise of catalase T activity during oxygen adaptation of the mutant cannot completely be excluded, but are rather improbable.

Although it is clear from the control experiments carried out with strain 26 B *rho*$^-$ that the *cgr*-1 mutation [12], which causes glucose repression-insensitive catalase T synthesis, is not sufficient to cause any accumulation of catalase T protein in the absence of oxygen, further genetic and biochemical investigations are also necessary to clarify whether this mutation is necessary for the loss of oxygen dependence of catalase synthesis. It seems interesting in this connection that recent studies by Biliński et al. (manuscript in preparation) have shown that another *cgr* mutant, *cgr*-4, is able to form active catalase T under anaerobic conditions.

Further experiments will also be necessary to decide whether the *cas*-1 mutation is pleiotropic, especially if it affects catalase A. Since glucose-grown *rho*$^-$ cells have no active catalase A and since the *cgr*-1 mutation influences only catalase T, such information would be difficult to obtain with the strain studied in this investigation. It will be therefore necessary to obtain strains having the mutation in a different genetic background.

The results of this investigation and of earlier studies seem to show that wild-type cells of *Saccharomyces cerevisiae* possess a regulatory mechanism or mechanisms whereby oxygen controls the synthesis or accumulation of the apoproteins of a number of heme proteins. Some proteins like cytochrome *c* peroxidase are not affected by this regulation. In our mutant, the mechanism controlling at least catalase T is apparently lost. Our results further show that *rho*$^-$ mutants that are defective in mitochondrial protein synthesis are able to respond to oxygen by synthesizing catalase T in amounts comparable to those formed in *rho*$^+$ strains. This demonstrates that an oxygen effect on mitochondrial translation as postulated [11] does not affect the formation of all heme proteins but is probably limited to the regulation of mitochondrially synthesized proteins. One can conclude therefore that not all regulatory effects of oxygen in *Saccharomyces cerevisiae* can be explained by one single mechanism.

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