BIOSYNTHESIS OF CATALASE T DURING OXYGEN ADAPTATION OF *SACCHAROMYCES CEREVISIAE*

Piotr ZIMNIAK, Engelbert HARTTER and Helmut RUIS

Institut für Allgemeine Biochemie der Universität Wien and Ludwig Boltzmann-Forschungsstelle für Biochemie, Wien, A-1090 Wien, Währingerstraße 38, Austria

Received 30 September 1975

1. Introduction

In recent years, the biosynthesis of mitochondrial heme proteins has been studied quite extensively in yeast and other organisms [1]. Comparatively less is known on the formation of heme proteins localized outside the mitochondria. The biosynthesis of catalase, an example of such an extramitochondrial heme protein, has been studied in rat liver by Lazarow and de Duve [2,3]. In the present investigation, such studies have been extended to *Saccharomyces cerevisiae*. This first report on the results of our work shows that catalase T, the major catalase protein of *S. cerevisiae* [4], is formed via a heme-less and a heme-containing precursor during oxygen adaptation of yeast grown on glucose under anaerobic conditions.

2. Methods

Catalase activity was determined spectrophotometrically as described by Bergmeyer et al. [5]. Catalase T and A ('typical' and 'atypical') from *S. cerevisiae* were purified to electrophoretic homogeneity essentially as described by Seah et al. [4,6]. Antibodies against both proteins were obtained by injection of the purified antigens into rabbits as described previously [7]. The antisera were shown to be monospecific by double diffusion according to Ouchterlony [8]. As has been reported previously [6,7], no immunological cross-reaction has been observed between catalase T and catalase A.

The haploid wild type strain D 273-10B was used for labeling studies. Cells were grown anaerobically on 0.3% glucose on a semisynthetic medium containing Tween 80 and ergosterol as described by Schatz and Kováč [9]. When cells were labeled with ^{59}Fe during oxygen adaptation, $FeCl_3$ was omitted from the medium during anaerobic growth. Immediately after cells grown anaerobically reached stationary phase ($A_{550} \sim 2.1$) 35 ml of the culture were transferred to a culture flask, labeled L-[^{3}H]leucine (100 μCi; 0.67 mCi/μmol) and $^{59}FeCl_3$ (100 μCi; 2.8 mCi/μmol) were added and oxygen adaptation was induced by rotatory shaking (180 rev/min). Samples of the culture were taken at appropriate time points, the cells were pelleted and washed twice with 0.05% L-leucine, 0.025% $FeCl_3 \cdot 6H_2O$, 0.01% trisodium citrate$\cdot 2H_2O$. Cells were broken in a Braun homogenizer after suspension in a medium consisting of 1% Triton X-100, 0.15 M NaCl, 0.05% L-leucine, 0.05% $FeCl_3 \cdot 6H_2O$, 0.08% trisodium citrate$\cdot 2H_2O$, 25 mM veronal–HCl (pH 8.2). Amounts of unlabeled catalase carrier appropriate for the following immunoprecipitation were added to the medium. The homogenate was centrifuged for 30 min at 48 000 rev/min in a Beckman 65 rotor. The supernatant of this centrifugation was filtered through a 0.2 μm membrane filter. For determination of total label incorporated into protein TCA* precipitable radioactivity of aliquots of the fractions was determined after treatment of the extracts with 0.1 N NaOH to hydrolyze charged leucyl-tRNA. Catalase T antigen was precipitated from the extracts by additon of appropriate amounts of antiserum determined by means of titra-

This paper is dedicated to Professor Karl Kratzl on the occasion of his 60th birthday.

*Abbreviations: TCA: trichloroacetic acid; SDS: sodium dodecyl sulfate.

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tion curves. After 12 h the immunoprecipitate was collected by centrifugation, washed with homogenization medium without catalase carrier, dissociated completely in 2.5% SDS, 10 mM dithiothreitol, 10 mM sodium phosphate (pH 7.0) and either counted in toluene–Triton X-100 scintillator or characterized by SDS gel electrophoresis using a system cross-linked with ethylene diacrylate [10]. Catalase T activity was determined in extracts prepared as described above from cultures without added label. Before the catalase assay catalase A present in the extracts was removed by quantitative immunoprecipitation [7].

3. Results

Extracts from cells of strain D 273-10B grown under anaerobic conditions to early stationary phase contain no measurable catalase activity, which means that these cells have less than 0.01% of the catalase activity of a stationary, fully oxygen adapted culture. No catalase T precursor recognized by the specific antiserum against this enzyme was detected by the Ouchterlony technique. Under our conditions the method would have been sensitive enough to detect 1% of the catalase antigen present in cells grown aerobically to stationary phase.

Immunoprecipitates from extracts of cells completely adapted to oxygen in the presence of L-[^{3}H]-leucine could be shown to contain a single radioactive product (fig.1). The mol. wt of this polypeptide as determined by SDS gel electrophoresis is 63 500, which corresponds well to the mol. wt of the catalase T monomer reported in the literature [4]. Products with identical mol. wt have been detected in earlier phases of oxygen adaptation after anaerobic growth. No distinct peak was obtained in immunoprecipitations carried out with non-immune sera.

Fig.2 shows the kinetics of incorporation of L-[^{3}H]-leucine and ^{59}Fe into catalase T immunoprecipitates during oxygen adaptation of a culture. Incorporation of both ^{3}H and ^{59}Fe label into the immunoprecipitate is clearly delayed compared to incorporation into total TCA-precipitable material. In the experiment shown incorporation of [^{3}H]leucine into the immunoprecipitate clearly precedes incorporation of ^{59}Fe, which itself precedes appearance of active catalase T. Whereas the delayed incorporation of ^{59}Fe compared to that of [^{3}H]leucine was consistently observed in a number of

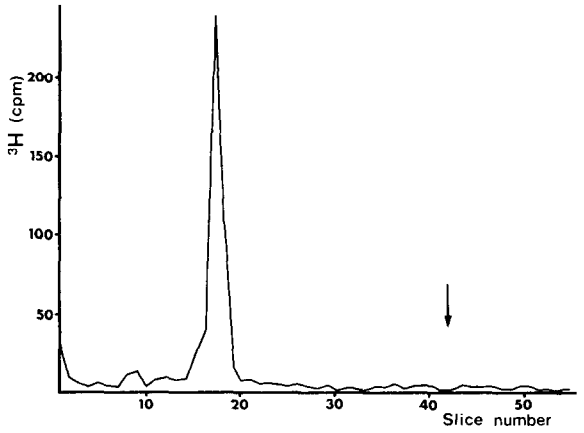

Fig.1. SDS gel of catalase T immunoprecipitate. Electrophoresis of L-[^{3}H]leucine-labeled immunoprecipitates dissociated with SDS was carried out as described in Methods. Gels were cut in 1 mm slices for counting. Arrow: bromphenol blue front.

such experiments, the delay in the formation of the active catalase compared to ^{59}Fe incorporation was variable and may respond to slight variations in growth conditions. When cells were completely adapted to oxygen, approximately 0.5% of ^{3}H and 2% of ^{59}Fe label of the TCA precipitable material was recovered in the immunoprecipitate.

At face value, the results obtained seem to show that catalase T is formed during oxygen adaptation via a heme-less precursor, which is then converted into an enzymatically inactive, or at least not fully active, heme-containing intermediate, which is finally converted to the active enzyme. However, since, for technical reasons, the label was added to the culture only when aeration was started, the possibility has to be considered that the results were distorted by a variation in intracellular specific radioactivities during the experiment. Labeling conditions used exclude the possibility of a significant decrease in specific radioactivities towards the end of the experiment. If a slow saturation of the L-leucine pool with radioactivity should have occurred during the first hours of oxygen adaptation this would be of no relevance to the qualitative conclusions drawn from the experiment. However, in the absence of a heme-containing intermediate, a delay in the incorporation of ^{59}Fe into the immunoprecipitate caused by a slow saturation of the intracellular iron pool with radioactivity could lead to

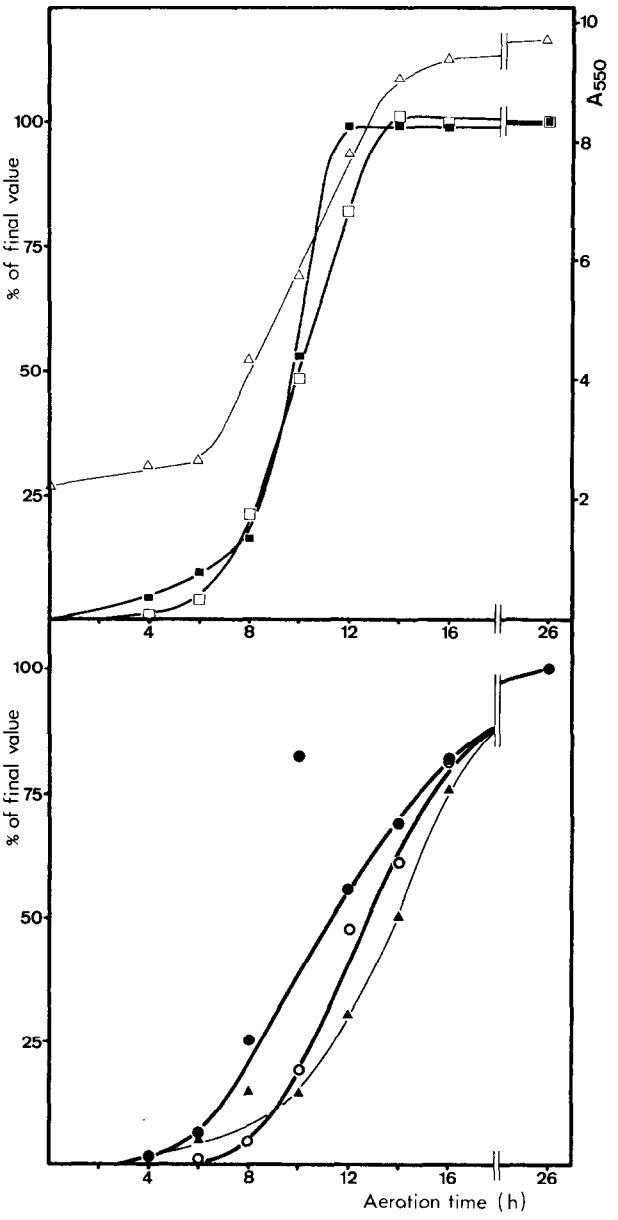

Fig.2. Formation of catalase T during oxygen adaptation. Growth of cells, preparation of extracts and immunoprecipitations are descibed in Methods. (■) ^{3}H in TCA precipitate, (□) ^{59}Fe in TCA precipitate, (●) ^{3}H in immunoprecipitate, (○) ^{59}Fe in immunoprecipitate, (▲) catalase activity. All values are normalized relative to the final value (100%). (^) growth of culture (A_{550}).

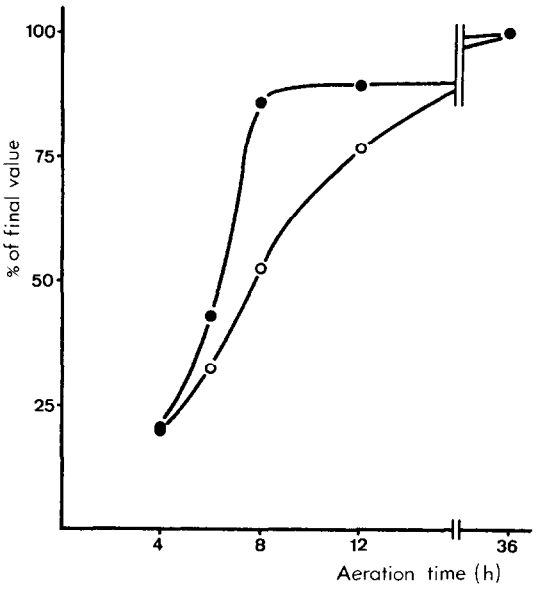

Fig.3. Incorporation of [^{3}H]leucine and ^{59}Fe into catalase T immunoprecipitates after prelabeling with ^{59}Fe during anaerobic growth. $^{59}FeCl_3$ was added to the culture at inoculation of the anaerobic culture, L-[^{3}H]leucine at the beginning of aeration. Preparation of extracts, immunoprecipitations and normalization are as in fig.2. (●) ^{3}H in immunoprecipitate, (○) ^{59}Fe in immunoprecipitate.

similar labeling kinetics as we have observed. Therefore, a control experiment was carried out where ^{59}Fe was added to the culture at the beginning of the anaerobic growth phase while [^{3}H]leucine was added again at the start of oxygen adaptation. As shown in Fig.3 even under such conditions ^{59}Fe is incorporated into catalase T antigen more slowly than [^{3}H]leucine. To avoid the use of an excessive amount of label in this experiment the cells had to be grown anaerobically in a different type of fermentor less suited for strict anaerobic growth. This led to a partial oxygen adaptation before additon or [^{3}H]leucine, which explains the high value of ^{59}Fe incorporated after 4 h.

Final proof for the existence of a heme-containing intermediate and additional evidence for a heme-less precursor of catalase T has been obtained in an attempt to demonstrate their presence in labeled extracts by gel chromatography (table 1). After separation on Sephadex G-150 the immunoprecipitate obtained from fraction I contains the active catalase while fraction II

Tabel 1
Fractionation of catalase T and its precursors on Sephadex G-150

Time h	Fraction	Immunoprecipitated radioactivity (cpm) ^{59}Fe	^{3}H	^{3}H/^{59}Fe	Fraction I/Fraction II ^{59}Fe	^{3}H
8	I	988	1112	1.13	13.0	3.2
	II	76	349	4.59		
16	I	16 148	14 567	0.90	21.0	22.5
	II	769	649	0.84		
42	I	23 182	20 489	0.88	174.3	103.0
	II	133	199	1.50		

Culture, labeling and extraction conditions were as described for fig.2. Extracts were separated on Sephadex G-150 superfine equilibrated with 0.15 M NaCl, 25 mM Veronal–HCl (pH 8.2). Catalase activity was used to locate fraction I, hemoglobin was added to extracts as a molecular weight standard for fraction II. Fractions were brought to concentrations of 1% Triton X-100, 0.05% $FeCl_3 \cdot 6H_2O$, 0.05% L-leucine, 0.08% sodium citrate·$2H_2O$, centrifuged at 48 000 rev/min in a Beckman 65 rotor and filtered through a 0.2 μm membrane filter. Quantitative immunoprecipitations were carried out as described under Methods.

should contain immunoprecipitable material with the molecular weight of a catalase T monomer. Although a rigorous quantitative estimation of the amount of precursors present in the extract is not possible because of some overlap in fractions and because of the possibility of a spontaneous tetramerization of monomers during the separation procedure the results shown in table 1 are in good qualitative agreement with those of fig.2. The ratio of ^{3}H to ^{59}Fe is fairly constant in the higher mol. wt fraction I throughout the experiment, which would be expected for a fraction containing only active catalase. In the lower mol. wt fraction II the ^{3}H to ^{59}Fe ratio is high after 8 h of aeration, indicating the dominance of a heme-less precursor, and comparable to that in fraction I after 16 h, which is in agreement with the almost exclusive presence of a heme-containing intermediate. The somewhat high value after 42 h may be due to an error caused by the fairly low absolute radioactivity present in this fraction. In agreement with the assumption that lower mol. wt precursors are converted into a higher mol. wt product the ratio of ^{59}Fe- and ^{3}H-radioactivities present in fraction I and II indicate a considerable shift of immunoprecipitable material from fraction II to fraction I between 8 and 42 h after beginning of aeration.

4. Discussion

In contrast to other organisms, *S. cerevisiae* contains two different catalase proteins, catalase T and A, which are probably biogenetically not directly related to each other (P. Zimniak, E. Hartter, unpublished results) and seem to differ from catalases of other organisms in their subcellular compartmentation [7]. The biosynthesis of both proteins is sensitive to glucose repression and completely dependent on the presence of oxygen. As this investigation shows, the yeast system, in contrast to rat liver, offers the possibility to study extensive net synthesis of catalases. In addition it will allow detailed investigations on the regulation of the biosynthesis of these proteins by oxygen and glucose. Our results show that catalase T is formed during oxygen adaptation via heme-less and heme-containing precursor molecules, which agrees well with results on the mechanism of biosynthesis of rat liver catalase [2]. It is quite probable, although not yet rigorously proved, that these two precursors are the apomonomer and the monomer of the enzyme. Further studies will have to deal with the identity of these precursor molecules. Our work is also being extended now to the biosynthesis of catalase A and to a more detailed investigation of the mechanisms

involved in the regulation of the biosynthesis of yeast catalases by oxygen.

Acknowledgements

We thank Professor Dr. O. Hoffman-Ostenhof for his interest in and support of this work. The investigations described have been supported by a grant from the Fonds zur Förderung des wissenschaftlichen Forschung, Wien, by the Hochschuljubiläumsstiftung der Stadt Wien and by the Hochschuljubiläumsfonds der Österreichischen Nationalbank.

References

[1] Schatz, G. and Mason, T. L. (1974) Ann. Rev. Biochem. 43, 51–88.
[2] Lazarow, P. B. and de Duve, C. (1973) J. Cell Biol. 59, 491–506.
[3] Lazarow, P. B. and de Duve, C. (1973) J. Cell Biol. 59, 507–524.
[4] Seah, T. C. M. and Kaplan, J. G. (1973) J. Biol. Chem. 248, 2889–2893.
[5] Bergmeyer, H. U., Gawehn, K. and Grassl, M. (1970) in: Methoden der enzymatischen Analyse, 2nd Edn. (Bergmeyer, H. U., ed.) pp. 388–483, Verlag Chemie, Weinheim.
[6] Seah, T. C. M., Bhatti, A. R. and Kaplan, J. G. (1973) Can. J. Biochem. 51, 1551–1555.
[7] Susani, M., Zimniak, P., Feßl, F. and Ruis, H. (1975) Eur. J. Biochem., submitted for publication.
[8] Ouchterlony, O. (1949) Acta Pathol. Microbiol. 26, 507–515.
[9] Schatz, G. and Kováč, L. (1974) Meth. Enzymol. 31, 627–632.
[10] Cain, D. F. and Pitney, R. E. (1968) Analyt. Biochem. 22, 11–20.